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TECHNICAL PROPOSAL

Responses to Short-term Fluctuations in Particulate Air Pollution in Asthmatic Children: Implications for Asthma Natural History

Part B:

Characterization of Asthmatic Children's Air Pollution Exposure in Fresno, California

Prepared for:

State of California Air Resources Board
Research Division
Sacramento, CA

Prepared by:

S. Katharine Hammond, Ph.D., CIH
School of Public Health
University of California
Berkeley, CA

October 1999

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Responses to Short-term Fluctuations in Particulate Air Pollution in Asthmatic Children: Implications for Asthma Natural History

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Characterization of Asthmatic Children's Air Pollution Exposure in Fresno, California

Principal Investigator:
S. Katharine Hammond, Ph.D.

Prepared for:

State of California Air Resources Board
Research Division
P.O. Box 2815
Sacramento, CA 95812

Prepared by:

S. Katharine Hammond, Ph.D., CIH
School of Public Health
140 Warren Hall
University of California
Berkeley, CA 94720-7360
(510) 643-0289
FAX (510) 642-5815
Email: hammondk@uclink4.berkelev.edu

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STATEMENT OF SIGNIFICANCE

The overall goal of this proposed study is to determine the effects of different components of particulate matter (PM), in combination with other ambient air pollutants, on the natural history of asthma in young children residing in the Fresno County region of California. Fresno County has an ethnically diverse population, a high prevalence of asthma, and high levels of ambient air pollution, especially PM, making it an appropriate location to address questions of how air pollution impacts this vulnerable population.

Gaseous pollutants and PM contribute significantly to asthma burden by causing acute asthma-related symptoms and short-term declines in lung function. However, an effective public health policy to protect asthmatics from the acute adverse effects of PM has not yet been achieved due to insufficient information about which pollutants, and at what concentrations and in what combinations of those pollutants, are associated with which observed effects. There are few data available to assess how day-to-day responses to air pollution affect long-term respiratory health and disease. A better understanding is needed of the biologic characteristics of asthma and exposure characteristics that define subgroups who are more or less responsive to sets of exposures or who experience larger effects associated with long-term exposures. This study proposes to fill many of these data gaps.

The study is comprised of two components: a panel studies component (PC) and a classical longitudinal component (LC). The PC will allow assessment of short-term exposure effects, which occur in different seasons (different air pollution and meteorological patterns). The protocols for the PC will be very similar to previous studies to facilitate comparison of results. The unique aspects of the PC will be the extensive exposure assessment program planned for this effort and the availability of high quality daily air pollution data obtained from two intensive air quality measurement efforts [the U.S. Environmental Protection Agency (EPA) "supersite" in Fresno and the California Regional $PM_{2.5}/PM_{10}$ Air Quality Study (CRPAQS) in the San Joaquin Valley]. The asthma study can be conducted much more cost-effectively by taking advantage of these air quality studies. To further enhance the cost effectiveness of the asthma study, measurements in schools and neighborhoods will be coordinated with other projects being initiated by the California Air Resources Board (CARB). The LC permits direct assessment of the effects of acute responses to air pollutants on the long-term behavior of asthma. Such an assessment has not been carried out in any previous study to date.

The depth and quality of air pollution measurements, exposure estimation, and detailed health evaluations in this five-year study will provide critical insights into the role of specific air pollutants (especially PM components) and other environmental factors in acute responses and the natural history of childhood asthma. This information can be used to evaluate ambient air quality standards and other air pollution-related public health policies and regulatory actions implemented at the state and community level. The study design allows assessment of host factors (biological and exposure-related) that influence susceptibility of subgroups of asthmatics; these insights potentially can lead to interventions that may be taken at the individual level. The combination of community- and individual-level action can lead to improvements in the protection of this highly vulnerable subgroup (i.e., asthmatic children) and to significant reductions in the direct and indirect asthma-related costs borne by all Californians.

ABSTRACT

The overall goal of this study is to determine the extent to which particle air pollution acts both independently and as a modifier of other environmental exposures (allergens, endotoxin infectious agents, etc.) to trigger asthma attacks and influence the occurrence of symptoms and the growth of lung function in asthmatics during the childhood years. Part B of this study is focused on characterizing the daily exposure of 450 asthmatic children to ozone, nitrogen oxides, $PM_{2.5}$ particles (mass, organic carbon/elemental carbon, sulfate ion, nitrate ion, ammonium ion, metals, potassium, PAHs, and particle number density), PM_{10} particles (mass, metals), environmental tobacco smoke (ETS), and an array of biological agents implicated in asthma, including pollens, fungal spores, endotoxin, and allergens in house dust (specifically, dust mites, cat dander, dog dander, and cockroach eggs).

A major hypothesis of this study is that pollutant concentrations vary spatially and pollutants can be systematically classified with respect to their spatial-scale of variability. Pollutants such as $PM_{2.5}$ sulfate, nitrate, and ammonium are expected to vary on the regional-scale. Many pollutants are expected to vary on the neighborhood-scale due to variations in local sources (traffic emissions of elemental carbon particles and nitrogen oxides. In addition, levels of ETS and allergens such as dust mites and cat dander are expected to be determined by household factors such as the presence of smokers, pets, and pests. The U.S. EPA "Supersite" in Fresno will provide daily data on PM mass/constituents/particle number density, and gaseous pollutants, to which we will add data on the biological agents. However, central site ambient air monitoring stations may not accurately measure the concentrations to which community residents are exposed. People who spend their time mostly indoors and, in some cases, closer to sources such as traffic, are likely to be exposed to levels different than those measured by the central site monitor. Therefore, a major thrust of the proposed research is to model personal exposure from measurements made at the central site, which will reflect temporal variability, combined with data on neighborhood factors, household-specific factors, and time-activity diary data indicating a child's location at different times of the day. Neighborhood variations will be evaluated from proximity to sources, such as traffic density; from measurements of key agents to be made at five sites in Fresno during the winter of 2000-2001 by CRPAQS; and from extensive measurements we will make inside and outside the homes of 96 subjects. During the health panel studies, we will sample for ozone, NO_2, and ETS for two weeks 2-4 times in each home over the first 2 years; house dust samples will be collected on each visit and analyzed for endotoxin and several allergens: dust mites, cockroach eggs, and cat and dog dander. The mobile vans (operated by the Air Resources Board) will be used in the second and third years to characterize the concentrations of air pollutants at schools. Questionnaire and diary data will be used to assess household characteristics, indoor pollution sources, and children's locations at various times of the day. These data will be combined in a model to characterize each child's daily exposure during the health study.

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1. INTRODUCTION

The overall goal of the combined studies (Part A and B) is to determine the effects of particulate matter (PM) air pollution, in combination with other ambient air pollutants, on the natural history of asthma in young children (ages 6 to 10 at intake) residing in the Fresno County region of California. This region of California is notable for a high prevalence of asthma among an ethnically diverse population and for high levels of ambient air pollution, especially PM and ozone. A unique opportunity to address critical questions related to air pollution's effects on the long-term progression of asthma is presented by two intensive air quality measurement efforts to be conducted in the study region. Specifically, the U.S. Environmental Protection Agency (EPA) "supersite" in Fresno and the California Regional $PM_{2.5}/PM_{10}$ Air Quality Study (CRPAQS) in the San Joaquin Valley. Part B of this study is designed to characterize the daily air pollution exposure of participants to selected pollutants in Fresno. It has been designed to capitalize on and enhance the intensive air monitoring studies. Part A of the study is designed to assess the health effects of selected air pollutants on these asthmatic children.

1.1 BACKGROUND ON THE AIR POLLUTION HEALTH EFFECTS STUDY – PART A

Asthma is a chronic airway inflammatory disease which is characterized by reversible airway obstruction, non-specific airway hyper-responsiveness, and mucus secretion. The occurrence of new asthma cases appears to be increasing (especially among children) in developed nations, posing a significant and costly public health problem. In California, an estimated 1.8 million people (or 1 in 20 Californians) have asthma, including a half million children. Asthma-related hospitalizations cost in excess of $350 million dollars in 1995, based on an evaluation by the California Department of Health Services (CDHS). This cost estimate does not include the direct and indirect costs associated with emergency room visits, medical office visits, prescriptions, days lost from school or work, or impacts on quality of life.

In the past decade significant progress has been made in the definition of the characteristics of asthma, especially its immunologic component. However, there is much to be learned about the etiology, natural history, and mechanisms underlying this disease. Among asthmatics, there is a wide spectrum of disease, ranging from mild to severe, with large individual variation with respect to how the disease progresses over time. Many factors may influence whether an individual develops asthma, the severity of disease once manifest, the triggers that cause asthma exacerbations (attacks) in an individual, and what course the disease takes over time (e.g., whether a mild case resolves, continues to be mild, or progresses to severe asthma). Genetic, environmental (including outdoor and indoor air pollution and allergens), dietary, and socio-cultural factors are under intensive investigation. In addition, the role of endotoxin, the lipopolysaccharide component of the cell wall of Gram-negative bacteria that is known to have potent inflammatory effects on the lung, has been raised. The specific role of each factor, as well as possible interactions among factors, is poorly understood with respect both to primary causation and to disease progression. Current research indicates that the factors and the underlying mechanisms for asthma onset and acute exacerbations of asthma (i.e., asthma "attacks") may be quite different.

There are two broad questions related to ambient air pollution's contribution to asthma-related disease burden in communities with biologically relevant levels of air pollution:

1. Do specific pollutants or combinations of pollutants, in the presence of other asthma risk factors, contribute to the increase in new cases of asthma?
2. For persons who have asthma, do ambient air pollutants contribute to exacerbations of asthma, and, if so, how does exposure to air pollution over the long-term affect disease progression?

It is critical that both questions are answered; however, this study will focus on the second question. Although one could potentially design a study capable of answering both questions, that design almost certainly would involve a study of longer duration, requiring significantly more resources, to achieve meaningful results. The decision to focus on the second question is based largely on considerations of the relative benefits to be gained from, and the resource requirements for, addressing each of the broad questions. Past studies have not provided compelling evidence that ambient air pollution is an important risk factor for induction of asthma, albeit those studies may not have been ideally suited to answer that question. There is substantial evidence that ambient air pollution causes exacerbation of asthma; however, due to limitations in environmental measurement and exposure assessment protocols, many questions remain regarding which pollutants are associated with specific effects. Few data are available to assess how day-to-day responses to air pollution affect long-term respiratory health or disease progression among a well-characterized (both in terms of health and exposure variables) population of asthmatics.

The two intensive air quality studies (Fresno Supersite and CRPAQS) have duration and intensities appropriate to serve as a basis for the health effects study; however, they are limited in three important aspects which Part B is designed to address. These limitations are that 1) not all air contaminants relevant to asthma are measured at these sites; 2) the spatial variability in the concentration of these air contaminants, especially the inside and outside home and home-specific factors, is ignored; and, 3) these are fixed location measurements and are not estimates of personal exposure. These health studies are well-suited to addressing questions related to how short-term and multiple year exposures to air pollution affect disease progression among individuals, specifically children, who already have diagnosed asthma. Within the context of the broad question noted above, the specific questions related to air pollution that this study will seek to answer are:

1. What is the relationship between short-term exposures to specific size fractions or constituents of particulate air pollution, or other ambient air pollutants. and acute exacerbations of asthma, which may include changes in lung function, occurrence of symptoms, and medications usage?
2. What are the critical exposures leading to the observed acute health effects? For example, at what concentrations are the effects occurring, is there an interaction with other outdoor and indoor pollutants (criteria pollutants, toxic air contaminants) or bioaerosols (pollens, spores, endotoxins), and can one identify specific sources of PM that are more strongly associated with specific adverse effects?

3. Are there cumulative effects of acute responses to short-term air pollution exposures that result in altered disease progression (e.g., asthma severity) or changes in other markers of health status (e.g., reduced lung function "growth")?
4. Among the general population of asthmatic children, what are the biologic characteristics (e.g., asthma severity, genetics, nutrition) or exposure characteristics (e.g., activity patterns, housing characteristics) that define subgroups who are more (or less) responsive to a given acute exposure or set of exposures or who experience larger effects associated with long-term exposures?

These questions are important and consistent with key issues and data gaps that the California Air Resources Board (ARB), the U. S. Environmental Protection Agency (EPA), and the National Research Council (NRC) have identified as high priority for current air pollution health effects research.

The study design for Part A involves following a cohort of asthmatic children for five years. A random sample of 450 clinically-diagnosed asthmatic children ages 6 to 10 in the Fresno/Clovis area will be enrolled from a register of asthmatic children provided by our community partners. This community has high asthma morbidity and a level of community organization around issues of asthma that made it an ideal site for such a study. Children will be enrolled in groups of 50; membership in each group will be fixed. The study design consists of a longitudinal component (LC) and a panel component (PC) with four and one-half years of follow-up. In the LC, each subject will undergo detailed baseline and six-monthly evaluations (medical history, house characteristics, medication use, lung function testing, prick skin testing, somatic growth). For the PC, each group of 50 subjects will be observed in ten 14-day panels (one in each of three air pollution seasons over four and one-half years). During panel periods, daily data will be obtained for forced expiratory volumes, symptoms, medication use, and time-location-activity patterns. Detailed air pollution data will be available from a central air monitoring station and from measurements made at the participants' homes, as described below. Extensive statistical analysis of the relationships between air pollutants and health outcomes will be performed to address the core study questions.

1.2 BACKGROUND AND HYPOTHESES FOR THE EXPOSURE STUDY – PART B

Numerous air pollutants are suspected of influencing the health of asthmatic children. Exposure to the "criteria" pollutants such as ozone, nitrogen dioxide (NO_2), and PM, to toxic mixtures of pollutants such as environmental tobacco smoke (ETS), and to biological contaminants such as pollens, spores, and endotoxins may influence the incidence, severity, and evolution of asthma in children. In this study, we plan to assess the exposure of asthmatic children in Fresno, California to all these contaminants. It is important to note that PM is a complex mixture; and when we refer to PM, we mean not only the mass of PM, but also its chemical composition and the particle sizes (or size distributions). In the absence of adequate mechanistic understanding of PM health effects, there is strong interest in determining which chemical components and/or particle size range may be associated with adverse health effects in sensitive children (if they exist).

The ambient pollutants of concern in this study are known to have, or are suspected of having, strong temporal variability (hour-to-hour, day-to-day, and season-to-season changes). This variability is primarily caused by fluctuations in meteorological conditions and is secondarily caused by the temporal variations of emissions. The ambient concentrations also vary spatially. The extent of spatial variation depends on the pollutant and proximity to source emissions. For most secondary pollutants, such as $PM_{2.5}$ sulfate, nitrate, ammonium, ozone, and secondary organics, ambient concentrations vary on a regional scale (i.e., 20-50 km between cities) rather than on an urban-scale (10 km) or neighborhood-scale (1-2 km) (Chow et al., 1992, 1998). Ambient concentrations of directly emitted species often have large spatial gradients near sources. Pollutants emitted by combustion sources [NO_x, SO_2, CO, $PM_{2.5}$ elemental carbon (EC), and $PM_{2.5}$ organic carbon (OC)] and resuspended PM from roadways, construction activities, and agricultural activities both are expected to have neighborhood-scale spatial variations. Likewise, the outdoor concentrations of pollens, fungal spores, and endotoxin are likely to depend on the local source strength and vary considerably across a city such as Fresno.

Human beings spend the majority of their time inside buildings. Even though children spend less time indoors than adults, children still spend, on average, 70 to 90 percent of their lives indoors. Pollutants of outdoor origin infiltrate buildings and coexist with pollutants emitted indoors. Indoor pollutant concentrations may depend on a large number of factors, including the types of indoor sources, indoor source use patterns, building air exchange rate, building volume and room design, type of HVAC system, types of surfaces, reactivity of pollutants, and concentrations immediately outside of the building. There are pollutants for which there are no significant indoor sources, such as ozone and $PM_{2.5}$ sulfate; the indoor concentrations of these species depend on outdoor concentrations, air exchange rates, and indoor loss rates (to deposition or chemistry). Indoor concentrations of other pollutants, such as ETS and house dust allergens, are almost solely determined by their indoor source strengths. Many common pollutants have indoor and outdoor sources that contribute to their indoor concentrations. Thus, another spatial scale of importance for exposure assessment is the residential scale. Realistic characterization of human exposure to pollutants of potential relevance for asthmatics must account for indoor-outdoor differences in exposure concentrations and the amount of time individuals spend in various microenvironments.

Knowledge of the spatial scales of pollutant variability is essential for characterization of exposures. The hourly and daily central-site air monitoring data that will be collected at the Fresno Supersite during this study are ideally suited to specification of the ambient concentrations of pollutant that vary only on the regional scale. Ambient concentrations of $PM_{2.5}$ sulfate, nitrate, ammonium, and secondary organics throughout the Fresno study area are expected to go up and down as indicated at the central site (Fresno Supersite). Ozone concentrations at many locations in Fresno are expected to be similar to those at the central-site monitoring station.

Ambient concentrations of pollutants with neighborhood-scale variability are expected to be biased from the central site yet often exhibit temporal variability similar to that at the central site. For example, ozone concentrations may be the same as the central site everywhere except near major roadways. Ambient NO/NO_2, $PM_{2.5}$ EC, $PM_{2.5}$OC, PM_{10} geologic material (road dust and soil dust), pollens, fungal spores, and endotoxin concentrations are likely to be modulated on the neighborhood scale by the local source strengths. Previous neighborhood-scale studies of

criteria pollutants in the San Joaquin Valley (e.g., Solomon et al., 1996; Ludwig, 1994) provide assurance that the within-community variations in species like PM_{10} and ozone are typically within ±30 percent of central-site measurements. Much less is known regarding the spatial variability of biological aerosols and other components of PM. There is tremendous diversity in vegetation throughout a city like Fresno, and large spatial variations in pollen releases are expected. The heterogeneity of pollen releases and their relatively short atmospheric lifetimes (before removal by gravitational settling) suggests there could be very significant local scale variability in these species. Too few ambient endotoxins measurements exist to know the appropriate spatial scale of variability. Likewise, ambient particle number densities are expected to be high near busy roadways and to fall off rapidly (more rapidly than primary gaseous pollutants) with distance from the roadway because of coagulation of the huge number of tiny particles emitted by motor vehicles. Such phenomena have not been adequately characterized in the neighborhood-scale studies, and new measurements are needed to understand the phenomena and to ultimately develop models to accurately estimate concentrations on these scales.

Certain pollutants will vary from house to house in a manner that strongly depends on the activities, operating characteristics, and materials in the individual houses. Important residential scale variations in concentrations must be captured with a combination of housing questionnaires and indoor and outdoor measurements at the homes of interest. New measurements are especially needed for the less frequently measured compounds such as pollens, fungal spores, endotoxins, house dust allergens, and other chemical components of indoor PM.

This conceptual model of pollutant variability in Fresno leads to the following hypotheses that are the central issues in the proposed study:

Hypothesis No. 1: The air pollutants of interest can be grouped into three categories on the basis of the principal determinants of their concentration.
- (a) Group I (regional pollutants)
- (b) Group II (neighborhood pollutants)
- (c) Group III (home-specific agents)

Hypothesis No. 2: Although regional pollutants vary over large distances (over >20 km), there is little variability of concentration within a region. These pollutants also have similar concentrations inside and outside a home. Among the pollutants which fall into this category are $PM_{2.5}$ sulfate, nitrate, ammonium, and secondary organic species.

Hypothesis No. 3: The primary determinants of the concentration of Group II agents are factors that vary from neighborhood to neighborhood. For instance, those pollutants whose primary source is traffic (e.g., EC) will be higher in neighborhoods near major traffic arteries than in those with little traffic. Similarly, pollen from particular plants will depend on the density of those plants in a neighborhood.

Hypothesis No. 4: Agents whose primary source are within the home fall into Group III. These agents include ETS, allergens from dogs and cats, fungal spores, dust mites, and cockroaches, and endotoxin.

Hypothesis No. 5. Daily air pollution exposure of individual children can be estimated (modeled) with reasonable accuracy from

(a) ambient air pollution concentrations measured hourly and daily at a central site in the community;
(b) representative samples of indoor and outdoor pollutant concentrations measured infrequently at a child's home;
(c) representative samples of the time activity patterns at known locations for each child, obtained during each two-week panel of health effects;
(d) housing and school characteristics determined from questionnaires;
(e) annual traffic densities on roadways near a child's home and school; and
(f) vegetation near a child's home and school.

1.3 SPECIFIC AIMS

The overall objective of Part B of the study is to estimate the daily air pollution exposures of the study participants during each of the two-week health panels over the five-year period with a high degree of reliability. The specific aims of the study are as follows:

1. To augment ambient air quality measurements at the Fresno Supersite with agents of interest in this study, specifically, pollens, fungal spores, and endotoxins.
2. To evaluate the daily variability of Group I (regional) and Group II (neighborhood) agents using the Fresno Supersite air quality data.
3. To measure the concentrations of Group I (regional) agents indoors and outdoors at selected homes and to evaluate their relationship to concentrations measured at the Fresno Supersite.
4. To develop definitions of neighborhoods based on traffic density and vegetation patterns.
5. To measure the concentrations of Group II (neighborhood) agents indoors and outdoors at selected homes, to evaluate their relationships to concentrations measured at the Fresno Supersite, and to assess the extent to which neighborhood parameters account for differences between neighborhood and Fresno Supersite concentrations.
6. To survey (by questionnaire and diary) home-specific factors for Group III (home-specific) agents.
7. To develop and test models to predict neighborhood-scale concentrations of the Group II (neighborhood) agents.
8. To develop and test models to predict the daily variability of Group III (home-specific) agents from measured data and diary data;
9. To measure the concentration of selected agents (ETS, NO_2, and ozone) in the home of each child during selected two-week health study panels.
10. To survey (by questionnaire and diary) the principal locations of the study participants on each day of each two-week health study panels.
11. To use the measurements made at the Fresno Supersite and homes and the questionnaire and diary data, as well as the models developed, to estimate the exposure of each child in

years of the study. Ozone measurements will be confined to the extended ozone season (May-September).

6. During the second year of the study, intensive air quality measurements will be made by UCB/STI during the panel studies at the homes of 96 participants. Six homes from each panel will be sampled for ozone, NO_2, light scattering by $PM_{2.5}$, $PM_{2.5}$ sulfate, $PM_{2.5}$ nitrate, $PM_{2.5}$ ammonium, $PM_{2.5}EC$, $PM_{2.5}OC$, PM_{10} mass, PM_{10} metals, PM_{10} endotoxins, pollens, spores, and ETS. All agents except ETS will be measured concurrently inside and outside the homes. Separate multi-day PM samples will be collected on weekdays and weekend days. Fifty-four houses will be sampled in two seasons. Another 42 houses will be sampled in one season.
7. A pilot-scale personal sampling program will be conducted by UCB/STI to collect NO_2, ozone, ETS, PM_{10} endotoxins, $PM_{2.5}$ mass, and PM_{10} mass on 25 participants during the two-week panel studies. Detailed time-activity diaries and household operating characteristics will be collected along with integrated 48-hr personal exposure samples.
8. Supplemental data will be provided by ongoing monitoring programs. Meteorological data will be provided by the National Weather Service (NWS) and ARB monitoring programs. Traffic count data will be provided for state and county roadways by CALTRANS.

All the measurements made by UCB/STI will be quality-controlled and quality-assured in accordance with generally accepted monitoring practices. All these data will be acquired and implemented in a Microsoft ACCESS database for use by all study participants.

The analyses of data collected in the study will first focus on characterization of the exposure concentrations to which the study participants are subjected, as indicated by measurements in their homes, schools, and from the central air monitoring site. These data will be carefully analyzed to characterize the within-community variability in concentrations of the different agents included in the study. We will identify the extent to which factors such as traffic density, neighborhood vegetation, housing characteristics, human time-activity patterns, and meteorology explain the observed variability in exposure concentrations. Relationships between agents will be fully explored to identify indicator species and metrics. We are especially interested in the relationships between the infrequently measured parameters and routinely collected parameters, and between biological agents and conventional pollutants.

The data will be analyzed to characterize relationships (1) between pollutant concentrations at the central site and those outside participants' homes and schools and (2) between indoors and outdoors at the participants' homes. The spatial scales of variability in concentrations will be ranked and categorized according to our three scales (regional, neighborhood, and residential). These analyses will lead to microenvironmental exposure models with parameters for each of the agents of concern in the study. The form of the models will depend on the spatial scale of specific pollutant variability. Model testing will be conducted using the personal sampling data. The database, models, and model estimates will be periodically delivered (in years 3, 4, and 5 of the study) to the health effects researchers so that they will be familiar with the data archive. The technical approach also involves closely monitoring how the data are used in the health analysis and review of the findings. The exposure team will provide feedback to the health analysis team to assure that data and model estimates are interpreted in appropriate ways.

the asthma health study to each agent of interest on each day during which the two-week health panels are conducted.

1.4 SUMMARY OF TECHNICAL APPROACH

Most epidemiologic investigations of associations between air pollution and health effects (1) rely on ambient air quality data from one or more central stations alone to assign exposures and (2) consider only criteria air pollutants. Typically, all individuals in a community the size of Fresno are assigned identical exposure values for ozone, NO_2, SO_2, CO, and PM_{10} mass on each day of the year. The technical approach for the proposed study is designed to overcome these limitations by using a more comprehensive approach to exposure assessment.

The technical approach for the exposure analysis will be to build databases and models to generate individual exposure estimates, rather than community average exposure estimates. The individual exposure estimates will be based on microenvironmental models adjusted for indoor, outdoor, and personal exposures and activity patterns. In addition, we plan to explore methods to adjust exposure assignments to account for proximity to roadways and traffic density because we expect these factors account for a significant portion of the within-community variation in ambient air quality.

The technical approach involves measurements of gases and both the chemical components and physical characteristics of PM that are beyond those conventionally measured for compliance monitoring. The groups of measurements incorporated into the study design are as follows:

1. The Fresno Supersite will provide measurements of ozone, NO/NO_2, SO_2, CO, particle number density, detailed particle size distributions, $PM_{2.5}$ mass, $PM_{2.5}$ sulfate, $PM_{2.5}$ nitrate, $PM_{2.5}$ ammonium, $PM_{2.5}EC$, $PM_{2.5}OC$, $PM_{2.5}$ metals, $PM_{2.5}$ polycyclic aromatic hydrocarbons (PAH), PM_{10} mass, and PM_{10} metals throughout the study period.
2. The ARB is sponsoring the development and deployment of mobile air monitoring vans that will make, at schools and in selected neighborhoods in Fresno, measurements of pollens, spores, PM_{10} endotoxins, ozone, NO/NO_2, SO_2, CO, particle number density, $PM_{2.5}$ mass, $PM_{2.5}$ sulfate, $PM_{2.5}$ nitrate, $PM_{2.5}$ ammonium, $PM_{2.5}EC$, $PM_{2.5}OC$, $PM_{2.5}$ metals, PM_{10} mass, and PM_{10} metals during the second and third year of the study.
3. UCB/STI will augment the central site with measurements of pollens, spores, and PM_{10} endotoxins. Samples will be collected throughout the study period. The samples collected in the first two years of the study will be analyzed in this program.
4. During the CRPAQS winter intensive study (December 1, 2000 through January 31, 2001), UCB/STI will augment four neighborhood air monitoring sites with daily measurements of pollens, spores, and PM_{10} endotoxins on the 15 intensive sampling days. These will be in addition to the measurements of $PM_{2.5}$, PM_{10}, ozone, and NO_2 made as part of CRPAQS at these neighborhood sites.
5. During the 2-week health panel studies, UCB/STI will collect integrated NO_2, ETS, and house dust allergen samples inside participants' homes and integrated ozone samples inside and outside participants' homes. These measurements will be made in the first two

1.5 PROJECT TEAM

The project team will be lead by Dr. S. Katharine Hammond. She will be assisted by Dr. Janet Macher, California Department of Health Services (CDHS), Dr. Paul Roberts, Sonoma Technology, Inc. (STI), and Mr. Fred Lurmann, Sonoma Technology, Inc. Other key personnel include Dr. Beth Wittig (STI), who will manage field operations in Fresno, and Dr. Steven Colome (UCLA), who will serve as a consultant on personal and microenvironmental monitoring techniques. The professional background and research interests of the project team leaders are described below. The investigators will be assisted by two other consultants and other staff at UCB and STI. The project team will interact frequently with Dr. Ira Tager, principal investigator for Part A of the study.

Dr. S. Katharine Hammond is an Associate Professor of Environmental Health Sciences at the School of Public Health, University of California, Berkeley (UCB). Dr. Hammond's role in the proposed study will be Principal Investigator, day-to-day program manager, and manager of all UCB laboratory analyses. She will be assisted by laboratory technicians, field technicians, data analysts, outside consultants, and graduate students. She holds a master's degree in Environmental Health Sciences and a doctorate in Chemistry. Her primary research is in exposure assessment for epidemiologic studies. She has developed new methods to sample for airborne chemicals, e.g., ETS. She has also developed models to evaluate exposure based on questionnaire data and validated these models with personal sampling measurements. She designed an exposure assessment strategy for a study of fiberglass workers exposed to a complex mixture of chemicals, among which extensive measurements were made of endotoxin, formaldehyde, and phenolics. She also designed and implemented the exposure assessment for a study of spontaneous abortion among semiconductor workers at fourteen companies across the United States. She has extensive experience measuring ETS in children's homes and developing sampling and laboratory methods for epidemiologic studies.

Dr. Janet Macher is an air pollution research specialist in the CDHS Indoor Air Quality Section. Dr. Macher will be a Co-Principal Investigator in the study and will oversee all of the biological sampling, laboratory analysis of biological components, and use of the biological agent data in the exposure and health analyses. She has a bachelor's degree in Biology from Ottawa University, a master's degree from the University of California, and a doctorate from Harvard University, the latter two with emphasis on industrial hygiene, public health, and microbiology. Dr. Macher has conducted research on engineering measures to control airborne infectious and hypersensitivity diseases (e.g., Legionnaires' disease and Pontiac fever). She is currently a co-investigator in a study at the University of Colorado at Boulder on the efficacy of ultraviolet irradiation to control the spread of tuberculosis. Other research includes evaluation of methods to collect and identify airborne biological agents (e.g., culturable and nonculturable microorganisms). Dr. Macher is co-author with Harriet Burge, Harvard School of Public Health, of a chapter on bioaerosol samplers in the ACGIH text, "Air Sampling Instruments." Dr. Macher is the past chair of the ACGIH Bioaerosols committee and co-editor of their 1999 publication, "Bioaerosols: Assessment and Control." These activities require familiarity with the health effects of biological agents and with currently available methods for their collection and analysis. She is also a collaborator on a project in Salinas, California, to measure exposures of neonates to pesticides, aeroallergens, and endotoxin. Many of the sample collection protocols and analytical procedures developed for the Salinas study will be directly applicable to the study of older asthmatic children in Fresno.

Dr. Paul Roberts is Executive Vice President and Manager of Regional Studies at STI. He will be a Co-Principal Investigator with responsibilities for the field measurements and exposure data management in the proposed study. Dr. Roberts holds bachelor's and master's degrees in Chemical Engineering from Rice University and a doctorate in Environmental Engineering Science from the California Institute of Technology. His areas of expertise include air quality and exposure assessment field measurements and associated data analyses and data management activities. He managed the installation and initial operation of the twelve routine monitoring sites and managed special field experiments, including ozone measurements in schools and personal ozone measurements, for the ARB-sponsored Children's Health Study in Southern California. He has designed and managed many large ozone and PM field studies throughout the United States, including studies in various locations in California, Texas, Louisiana, the Gulf of Mexico, the area around Lake Michigan, and the northeastern United States He is currently the Technical Coordinator for the CRPAQS anchor-site field measurements in the San Joaquin Valley. Dr. Roberts was a member of the California Inspection and Maintenance Review Committee during 1994-1995 and has been on various EPA peer-review panels since 1995.

Mr. Fred Lurmann is President and Manager of Exposure Assessment Studies at STI. He will be a Co-Principal Investigator responsible for exposure data analysis and model development. Mr. Lurmann received his master's degree in Mechanical and Environmental Engineering from the University of California, Santa Barbara. Mr. Lurmann has been involved in the field of air pollution modeling for 22 years. He has served on a number of exposure assessment and air quality modeling review committees since the mid-1980s, including the ARB's Modeling Advisory Committee and the South Coast Air Quality Management District's Modeling Working Group. Mr. Lurmann's research interests are in urban and regional air quality modeling, atmospheric chemistry, aerosol modeling, and human exposure assessment. He has been involved with the development of a number of air quality models (UAM-AERO, RADM, SAQM-AERO, ADOM, PLMSTAR) and directed modeling applications in California and elsewhere in the United States. In addition, he developed the Regional Human Exposure (REHEX) model that has been applied to Los Angeles, San Francisco, and Houston for general population exposure assessment to PM, ozone, NO_2, and benzene. Through his work in exposure assessment, Mr. Lurmann is familiar with exposure measurement studies, exposure databases, and exposure models. He has been responsible for the exposure assessment components in numerous epidemiologic studies, including the ten-year ARB/USC Southern California Children's Health Study of chronic air pollution effects. Mr. Lurmann has developed Monte Carlo exposure models to estimate the daily exposures of epidemiologic study participants from ambient air quality data, indoor-outdoor relationships, and time-activity and housing survey data.

Dr. Beth Wittig will coordinate the field measurements for the exposure (Part B) portion of the study. Dr.Wittig holds a bachelor degree from UCLA and a doctorate from the University of Texas at Austin, both in Chemical Engineering. Her areas of expertise include the design and coordination of air quality field studies and the validation, quality control, and analysis of air quality data. In this study, she will be responsible for the field installation and operations, including sampling logistics, sampling and calibration strategies, instrument checkout and installation, instrument trouble shooting, and operator training and supervision. These activities are similar in scope and magnitude to her current activities for the CRPAQS. In addition, Dr. Wittig will be able to coordinate the startup of the proposed measurements while coordinating the shutdown of the CRPAQS.

Steven D. Colome is an adjunct professor at the University of California, Los Angeles, School of Public Health. His role in the project will be as a consultant on personal and indoor sampling methodologies. Dr. Colome's research is in the areas of human exposure assessment, environmental epidemiology, indoor air quality, regional exposure modeling, and health effects assessment. Dr. Colome brings to the project more than 20 years of experience with air quality and exposure assessment, much of which has been in California. He has conducted numerous small-scale and large-scale studies of indoor, outdoor, and personal exposures for CO, NO_2, ozone, and $PM_{2.5}$, and PM_{10}. He has published extensively on the relationships between personal exposure and both indoor and outdoor air quality.

Resumes of key personnel are included in Appendix A.

1.6 ORGANIZATION OF PROPOSAL

The following sections of this proposal provide background for and descriptions of this unique exposure assessment study. Section 2 provides background information on air quality conditions in Fresno and the biological aerosols of concern in this study. Section 3 describes the technical approach and rationale for the methods, measurements, data management, data analyses, and quality control/assurance that will be employed in the study. Section 4 provides a succinct statement of work that will be conducted under the sponsorship of this study. The project management, organization, key personnel, and schedule are provided in Section 5. References are provided in Section 6. Included in the appendices are resumes of key personnel, sample standard operating procedures for selected laboratory analyses, and a list of supplies and equipment assumed to be provided by Part A of the study.

1.7 REFERENCES

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2. BACKGROUND

Numerous air pollutants are suspected of influencing the health of asthmatic children. Exposure to the "Criteria" pollutants, such as ozone, nitrogen dioxide (NO_2), and particulate matter (PM), and biological contaminants, such as pollens, spores, and endotoxins, may influence the incidence, severity, and evolution of asthma in children. In this study, we plan to assess the exposure of asthmatic children in Fresno, California to all of these contaminants. It is important to note that PM is a complex mixture, and when we refer to PM, we mean not only the mass of PM, but also its chemical composition and the particle sizes (or size distributions). In the absence of adequate mechanistic understanding of PM effects, there is strong interest in determining which chemical components and/or particle size range may be associated with adverse health effects in sensitive children.

Pollutant concentrations are known to vary spatially and temporally and among different microenvironments, such as indoor residences, schools, vehicles, shopping malls, etc. Characterization of the exposure of children to these different chemical agents requires an understanding of the nature of variations in their concentrations. In fact, any method of estimating daily exposure levels of individuals within a community incorporates explicit or implied assumptions about the nature and sources of this variability. Though patterns of man-made emissions vary between weekdays and weekends and among different hours of the day, the general pattern of emissions is repetitive. Overlaid on the general temporal/diurnal patterns of man-made species emissions are distinct seasonal variations for emissions that are influenced by temperature, moisture, and vegetation growth state. For example, emissions of bioaerosols, biogenic hydrocarbons, and wind blown dust are likely to have large seasonal variations. Pollutants primarily emitted by indoor sources may exhibit patterns that are totally unrelated to the patterns of outdoor pollutants. Yet, these pollutants of indoor origin are likely to have some common seasonal characteristics (e.g. greater number of spores in winter, indoor PM in winter from wood smoke).

The spatial variability in pollutant concentrations depends on the heterogeneity of emission patterns, proximity to emission sources, atmospheric chemistry, transport patterns, and modifying effects of structures. For purposes of planning this study, we identify three principal spatial scales of variability in exposure concentrations:

1. Variations between regions
2. Variations between neighborhoods in a given region
3. Variations between residences, schools or other buildings in a neighborhood[1]

Most of the pollutants of interest in this study can be classified with respect to their predominant spatial scale of variations. **Table 2-1** shows the expected scales of variation for the different pollutants. Numerous pollutants vary on multiple scales. The basis for these scales is described in the subsequent sections.

[1] One can also consider the room-to-room variations within residences, schools, or other buildings, but this is impractical for any realistic assessment because, even if one could afford to characterize exposures in every room, tracking the movements of study participates between rooms for five years is not feasible.

Table 2-1. Principal spatial scales of variations in pollutant concentrations.

Pollutants that Tend to Vary on the Regional-Scale	Pollutants that Tend to Vary on the Neighborhood-Scale	Pollutants that Tend to Vary on the Residential Scale
Ozone	Ozone[a]	NO/NO_2
$PM_{2.5}$ SO_4	NO/NO_2	Fungal spores
$PM_{2.5}$ NO_3	Pollens	PM_{10} Endotoxins
$PM_{2.5}$ NH_4	Fungal Spores	$PM_{2.5}$ Mass
SO_2	PM_{10} Endotoxins	$PM_{2.5}$ OC/EC
	$PM_{2.5}$ Mass	$PM_{2.5}$ NO_3
	$PM_{2.5}$ OC/EC	$PM_{2.5}$ NH_4
	$PM_{2.5}$ PAH	$PM_{2.5}$ PAH
	$PM_{2.5}$ Metals	$PM_{2.5-10}$ Mass
	$PM_{2.5-10}$ Mass	$PM_{2.5-10}$ OC
	$PM_{2.5-10}$ OC	$PM_{2.5-10}$ Metals
	$PM_{2.5-10}$ Metals	Particle Number Density
	PM_{10} Latex	Allergens (from dogs, cats, dust mites, and cockroaches)
	Particle Number Density	

a) Ozone near heavily traveled roadways is expected to vary on the neighborhood scale.

2.1 AIR QUALITY CHARACTERISTICS OF FRESNO

Fresno is the largest city in the San Joaquin Valley and often exhibits the valley's poorest ambient air quality. The factors that contribute to adverse air quality in Fresno include (1) meteorological conditions that favor recirculation and buildup of pollutants, (2) its relatively large urban population (400,000 in Fresno, 65,000 in neighboring Clovis, and 300,000 in other parts of Fresno County in 1996), (3) its central location in the valley, (4) its proximity to major highways (Highways 99 and 41), and (5) the extensive agricultural and industrial operations in the area. Compared to other similarly sized cities, Fresno has relatively high pollutant levels. Fresno's population and economy are growing fast, and the concern is that the trend in ambient pollution levels has shown smaller reductions in Fresno than in other urban areas in California.

2.1.1 Criteria Pollutant Concentrations and Emissions

Ambient air quality has been monitored for compliance purposes at several locations in Fresno County since the late 1970s. **Figure 2-1** shows a map of the roadways and current routine air monitoring station locations in Fresno. The routine monitoring focuses on the criteria air pollutants (ozone, NO_2, PM_{10} mass, and $PM_{2.5}$ mass) for which there are National Ambient Air Quality Standards (NAAQS) and/or California air quality standards. The routine monitoring

site located at First Street will become an EPA supersite with greatly enhanced monitoring in the year 2000. The First Street monitoring station will provide the detailed daily air quality data needed for the five-year health/exposure study. Note that the First Street station is centrally located in Fresno.

Ozone

Fresno has relatively high ozone concentrations. The 80 ppb 8-hr daily maximum ozone NAAQS and 120 ppb 1-hr NAAQS are exceeded on numerous days each summer in Fresno and Clovis. On the worst days of the year, the maximum concentrations are 20 to 30 percent above the Federal standards. In a bad year, the 8-hr daily maximum standard may be exceeded on as many as 75 days in Fresno or Clovis. Emission control programs have been implemented to reduce volatile organic compound (VOC) and NO_x precursor emissions in the San Joaquin Valley, Sacramento, and the San Francisco Bay Area; these programs are expected to gradually reduce ozone levels in the coming years.

For purposes of this health/exposure study, we are concerned about persistent spatial patterns of concentrations within the study area. Long-term average concentrations provide insight to such patterns. **Figure 2-2** shows the annual average ozone concentrations for 1992-1995 at the three locations in Fresno and one station in Clovis. **Figures 2-3 and 2-4** show the annual average 0010-1800 PST ozone and 1-hr daily maximum ozone at these same locations. For these years, the annual concentrations are remarkably similar at Sierra Sky Park in the northwest corner of the study area, at Clovis in the northeast corner of the study area, and at First Street in the center of the study areas. The 0010-1800 PST average ozone levels are about 50 ppb in these three neighborhoods. The annual ozone concentrations are 10 to 20 percent lower at the Drummond Street station than at the other three stations in the region. The Drummond station is located south of central Fresno and is closer to (and downwind of) the heavily traveled Highway 99 than the other stations. The ozone concentrations at these stations are highly correlated. For example, correlation between 0010-1800 PST daily ozone concentrations at First Street and the other three stations is 0.97 to 0.98. These data support the notion that, while ozone has strong diurnal and seasonal variations at all of these stations, there appears to be significant spatial homogeneity on an annual basis in Fresno. We expect ozone levels to remain highly correlated throughout the community, and we expect a negative bias at locations that are close to heavily traveled roadways. The neighborhood scale variations are expected to occur because of local scavenging of ozone by the high NO emissions that occur near busy roadways.

Ozone concentrations in residences and schools are expected to be low under most circumstances. There are virtually no indoor sources of ozone, and numerous studies have shown that indoor ozone levels are almost always lower than outdoor levels because ozone deposits rapidly on many indoor surfaces. Avol et al. (1996) found indoor-to-outdoor ozone ratios of 0.37 ±0.19 in 125 Southern California homes. Lurmann et al. (1994) found indoor-to-outdoor ozone ratios of 0.28 ±0.17 in 48 Southern California schools. Air conditioning tends to dramatically reduce indoor ozone levels (because of low air exchange rates) while buildings with open windows tend to have similar indoor and outdoor ozone levels (because of the high air exchange rates). There is a need to characterize ozone indoor/outdoor ratios of

individual homes in the summer months to be able to estimate indoor exposures from central-site, outdoor ozone data.

Nitrogen Dioxide

Ambient concentrations of nitrogen dioxide (NO_2) are in compliance with the annual average NAAQS (0.050 ppm) and the 1-hr daily maximum California air quality standard (0.25 ppm). **Figure 2-5** shows the annual average NO_2 concentrations observed at the First Street, Clovis, Drummond Street, and Sierra Sky Park stations from 1992-1995. These long-term concentrations indicate that the First Street and Drummond Street neighborhoods have very similar NO_2 levels most years (around 22 ppb in 1995). The Clovis suburb has levels that are 10 to 20 percent lower than those at First Street and Drummond Street. The Sierra Sky Park area has concentrations that are 20 to 30 percent lower than those at First Street and Drummond Street. The correlation of daily NO_2 concentrations at First Street with those at Clovis, Drummond, and Sierra Sky Park are 0.97, 0.87, and 0.80 in 1995, indicating concentrations generally move up and down together across the community. Fresno NO_2 concentrations tend to be higher in the fall and winter than in the spring or summer.

NO_2 is directly emitted by outdoor combustion sources (2 to 10% of NO_x emissions) and is rapidly formed in the atmosphere by the reaction of fresh NO emissions with background ozone. Near roadways, ambient NO_2 concentrations often show elevated morning and evening concentrations that reflect the contributions from commuter traffic. Like other criteria pollutants, the day-to-day and seasonal variations in NO_2 are primarily controlled by meteorological variability and secondarily by day-of-week variations in emissions, especially from weekday-weekend differences in mobile sources. We expect there will be significant within-community variability in ambient NO_2 concentrations in the greater Fresno area. Proximity to mobile sources is the primary factor expected to cause the neighborhood scale variability in NO_2. Proximity to stationary combustion sources could also be a factor in certain portions of the community.

There are a number of indoor sources of NO_2. Unvented gas appliances are a common source of NO_2 in residences. Many studies have shown that gas ranges can increase indoor NO_2 concentrations above ambient levels (Samet and Spengler, 1991). The NO_2 levels in homes with gas ranges are nearly always higher than levels inside homes with electric ranges in the same communities (Yocum, 1982). Both continuous pilot lights and cooking on gas ranges add significantly to the indoor NO_2 levels. Ryan et al. (1998) found continuous pilot lights contributed an additional 12 ppb of NO_2 indoors, which is about half of the outdoor ambient levels in Fresno. Surveys of indoor NO_2 concentrations show considerable variations among homes, reflecting differences in source use, emission rates, air exchange rates, housing volume, and mixing of the air. Homes with wall and floor furnaces were found to have elevated NO_2 in Southern California, while public housing units had elevated NO_2 in Boston. While it is possible to classify homes for potential indoor NO_2 levels from housing surveys, it is more reliable to classify them based on actual NO_2 measurements in the houses of interest.

Particulate Matter

Ambient concentrations of the mass of particles with aerodynamic diameters less than 10 μm (PM_{10}) in Fresno indicate that the greater Fresno area has been in compliance with the 24-hr maximum (150 $\mu g/m^3$) and annual average (50 $\mu g/m^3$) PM_{10} NAAQS since 1994. **Figure 2-6** shows that the annual average PM_{10} mass concentrations have decreased from around 60 $\mu g/m^3$ in 1990-91 to around 40 $\mu g/m^3$ in 1996-1997. PM_{10} levels at the First Street monitor are consistently higher than those at the Clovis monitor and lower than those at the Drummond Street monitor. However, the differences among monitoring areas are not large on an annual basis; the annual PM_{10} levels at the other sites are within ±5 $\mu g/m^3$ of those at First Street (except in 1994). Daily PM_{10} concentrations at Drummond Street and Clovis correlate reasonably well ($R>0.90$) with those at First Street.

$PM_{2.5}$ mass data collected with a Dichotomous sampler at the First Street stations suggest that the 65 $\mu g/m^3$ 24-hr NAAQS and the 15 $\mu g/m^3$ annual NAAQS are exceeded in Fresno. While the Dichotomous sampler is not the official Federal reference method for assessing compliance with the standard, the $PM_{2.5}$ data are high enough to warrant concern for $PM_{2.5}$ exposures of sensitive subgroups in Fresno. As shown in **Figure 2-7**, the annual average $PM_{2.5}$ mass concentrations varied from 16 to 26 $\mu g/m^3$ between 1991 and 1997 at the First Street monitor. The daily $PM_{2.5}$ mass concentrations are moderately well-correlated with the PM_{10} mass, and the $PM_{2.5}$ levels are 43 percent of the PM_{10} levels on average. That is, there is generally more coarse mass than fine mass in Fresno, but the relative amounts of fine and coarse material changes with the seasons. The $PM_{2.5}$ is a relatively large fraction of PM_{10} in winter and a relatively small fraction of PM_{10} in summer.

The chemical composition of PM in Fresno is similar to that in other urban areas in California. **Table 2-2** shows the average chemical composition of fine and coarse PM in Fresno during the warm (summer) and cool (winter) seasons of the year. These data are from the 1988-89 Valley Air Quality Study, which was one of the few year-round PM sampling programs conducted in Fresno (Chow et al., 1992). The $PM_{2.5}$ composition is dominated by directly emitted carbonaceous species, secondary inorganic species, and secondary organic species. Geologic material is only 5 to 10 percent of the $PM_{2.5}$ mass. In the warm season, about 66 percent of the fine PM are accounted for by sulfate, nitrate, ammonium, EC, and organic material. In the cool season, these components account for 71 percent of the fine PM on average. Although the relative composition of $PM_{2.5}$ is similar in the warm and cool seasons, the $PM_{2.5}$ mass is about three times higher in the cool season. The more recent $PM_{2.5}$ chemical composition data from the 1995 Integrated Monitoring Study (IMS95) show similar characteristics for winter conditions in Fresno (Kumar et al., 1998). The secondary organic aerosols are probably significant in all seasons (Strader et al., 1998). The IMS95 data show that ammonia is exceedingly abundant in the San Joaquin Valley, and the sulfate and nitrate are fully buffered by ammonium (Kumar et al., 1998). We are not aware of any measurements of fine aerosol acidity (H^+) in the valley, and, given the abundance of ammonia, we would not expect to be able to measure aerosol acidity (other than in dilute fog droplets).

As shown in Table 2-2, the $PM_{2.5-10}$ chemical composition is dominated by geologic material, which include oxides of silica, iron, aluminum, etc. Sulfate, nitrate, ammonium, and elemental carbon account for only 3 to 4 percent of the coarse PM mass. Note that significant

amounts of organic material (20-30 percent of mass) are present in the coarse and fine fraction throughout the year. However, the key metals of interest for the health effects study are primarily in the coarse fraction. The 1991-97 Fresno Dichot XRF data indicate, for example, that 85 percent of the iron is in the coarse fraction. The coarse PM mass is about 60 percent lower in the cool season than the warm season in Fresno.

Chow et al. (1992) applied the Chemical Mass Balance (CMB) model to estimate the sources responsible for PM in Fresno. On an annual basis, the estimated $PM_{2.5}$ source attribution in Fresno was 34 percent from secondary components, 19 percent from directly emitted motor vehicle exhaust, 12 percent from vegetative burning, 5 percent from geologic material, and 31 percent from other unidentified sources. The estimated annual PM_{10} source attribution includes 44 percent from geologic material, 21 percent from secondary components, 10 percent from directly emitted motor vehicle exhaust, and 18 percent from other source types. The measured chemical composition and source attribution estimates confirm the notion that the PM in Fresno is a complex mixture that cannot be realistically characterized by mass alone.

Table 2-2. Fine and coarse PM chemical composition in Fresno during the 1989-90 warm and cool seasons (from Chow et al., 1992).

Component	April - September		October - March	
	$PM_{2.5}$	$PM_{2.5-10}$	$PM_{2.5}$	$PM_{2.5-10}$
Nitrate	9%	1%	22%	0%
Sulfate	11%	1%	4%	2%
Ammonium	7%	0%	9%	0%
Elemental Carbon	9%	1%	13%	2%
Organic Material[a]	30%	20%	23%	30%
Aluminum	1%	12%	0%	7%
Silica	3%	30%	0%	18%
Calcium	1%	3%	0%	2%
Iron	1%	5%	0%	4%
Other	28%	27%	28%	35%
Mass ($\mu g/m^3$)	23.9	38.6	66.1	23.8

a) Organic material is estimated as 1.5 times organic carbon.

2.1.2 Emissions

The ARB's estimated emissions of PM_{10}, NO_x, reactive organic gases (ROG), and SO_x in Fresno County in 1996 are shown in **Table 2-3**. The principal sources of primary PM_{10} emissions in the inventory are farming operations, road-dust, and other area sources. Stationary source fuel combustion and on-road and off-road motor vehicles are the predominant sources of

NO_x. Motor vehicles, along with other stationary and area sources, are estimated to contribute the majority of ROG emissions. These ROG emission estimates do not include biogenic hydrocarbons from vegetation, which are estimated to be large in the growing seasons. Emissions of SO_x are quite small compared to those for the other species and are primarily from stationary fuel combustion sources. It should also be noted that air quality conditions in Fresno are significantly influenced by emissions occurring outside Fresno County (including other portions of the San Joaquin Valley, San Francisco Bay Area, Sacramento, central coast region, and southern California).

These emission estimates should be interpreted cautiously because they are based on uncertain estimation procedures. A number of recent emission inventory evaluation studies have suggested many emission inventories overestimate dust emissions and underestimate motor vehicle particle and ROG emissions (Watson et al., 1998). A principal objective of the CRPAQS is to characterize the nature and causes of PM concentrations in and around central California. CRPAQS will provide better emissions information and reconciliation of emissions and ambient air quality data.

Table 2-3. Estimated air emissions in Fresno County in 1996.

Category of Emissions	Annual Emissions (tons/day)[a]			
	PM_{10}	NO_x	ROG	SO_x
Stationary Sources – Fuel Combustion	2.8	28	0.7	4.9
Stationary Sources – Other	2.9	6	21.3	3
Area Sources – Farming Operations	33		7.4	
Area Sources - Road Dust	50			
Area Sources – Other	37	3.3	25.6	0.2
Mobile Sources – On-road Gasoline Vehicles	0.6	38.1	43.1	0.3
Mobile Sources – On-road Diesel Vehicles	0.9	11.9	1.3	0.5
Mobile Sources – Off-road Vehicles	0.8	14	6.7	0.4
Natural Sources	2.5	0.2	1[b]	0
Total	130	100	100	9.3

a) Source: California Air Resources Board, 1998
b) Biogenic hydrocarbon emissions are not included in this inventory.

2.2 NON-CRITERIA POLLUTANTS AND BIOLOGICAL POLLUTANTS

Bioaerosols are those airborne particles that are living or that originated from once-living organisms. Individual bioaerosols range in size from submicroscopic particles (<0.01 μm) to particles greater than 100 μm in diameter. Included in this category are microorganisms and fine fragments from plants, animals, and microorganisms. Bioaerosols may become airborne individually (e.g., pollen grains and some fungal spores) or in association with particles from soil, plants, and animals. Endotoxin is a term for a toxin in the outer membrane of Gram-negative bacteria. Gram-negative bacteria and endotoxins are found widely in soil and water and in association with animals and plants. Also considered bioaerosols are particles of dander, saliva, and urine from domestic and wild animals (e.g., cats, dogs, rodents, and birds) and fragments and excreta from arthropods (e.g., house dust mites and cockroaches).

Bacterial and fungal cells, spores, and fragments are released into the air by natural means (e.g., spore dispersal) and physical disturbance of contaminated materials. Bioaerosols are ubiquitous in nature, and all persons are exposed daily to a wide variety of such materials from indoor and outdoor sources. The coarse particle fraction (>2.5 μm) is dominated by material from natural sources, such as fugitive and resuspended soil, pollen, fungal spores, and bacteria. Bioaerosols are of concern because of their potential infectivity and allergenicity as well as their inflammatory and toxic effects (Institute of Medicine, 1993; Lacey and Dutkiewicz, 1994; Rose, 1994a, 1994b; Rose et al., 1999; Rylander and Jacobs, 1994; Wald and Stave, 1994; Cookingham and Solomon, 1995; Horner et al., 1995; Platts-Mills et al., 1995; Levetin, 1997; Platts-Mills and Carter, 1997; Platts-Mills et al., 1997; Sattar and Ijaz, 1997; Yang and Johanning, 1997; ACGIH, 1999).

Concentrations of bioaerosols in ambient air and indoors are not frequently measured in air pollution exposure studies. Existing data are insufficient to characterize the population's exposures to these compounds in Fresno. Pollens and spores have been measured and counted by allergists in Fresno (at a location about one mile from the central site); however, these data are not archived. Data for endotoxins are virtually nonexistent in the region. Hence, there is no substitute for collecting both central-site data and data in the children's homes to characterize the levels to which they are exposed.

2.3 ESTIMATION OF PERSONAL EXPOSURES

A fundamental assumption of most air pollution epidemiologic studies is that concentrations of outdoor pollutants are an adequate surrogate for personal exposure as well as dosage. Carefully designed total exposure assessment studies for CO (Akland et al., 1985), VOCs (Wallace et al., 1991), and PM (Wallace, 1996; Ozkaynak et al., 1996) have provided a more complete understanding of the relative contributions of outdoor pollutants to total personal exposure. It is now well known that for many pollutants, even those for which the major sources are outdoors, there is substantial variability in population exposures. Among the factors contributing to variability in personal exposures are the impact of indoor sources, the time-activity profiles (i.e., the amount of time individuals spend in different microenvironments), and the spatial variability in ambient concentrations. As research has progressed, it has become clear that in many instances ambient monitoring at fixed locations may not adequately or

2.3.2 Estimating Personal and Indoor Exposures

A variety of physical and statistical modeling methods have been developed for predicting exposures to gases and particles (see for example Duan 1982; Ott 1985; Lurmann et al., 1989; Ryan, 1991). The common feature of these models is that they employ the microenvironmental approach where the total exposure (E_t) is modeled as the sum of time-activity weighted exposures encountered in various microenvironments. Daily personal exposures (E_i) are estimated from

$$E_i = \sum_{j=1}^{m} E_{ij} = \sum_{j=1}^{m} f_{ij} C_{ij} \qquad (2\text{-}1)$$

where

E_{ij} = exposure of individual *i* in microenvironment *j*
f_{ij} = fraction of time spend by person *i* in microenvironment *j* during the 24-hr prediction period
C_{ij} = average concentrations in microenvironment *j* when individual *i* is present
m = number of microenvironments considered in the model.

Often the number of microenvironments that can be distinguished is small. Common applications for children might consider five microenvironments: (1) outdoors at home, (2) indoors at home, (3) indoors at school, (4) outdoors at school, and (5) in transit. Obviously, it is important to obtain subject-specific time-activity information to accurately partition time between locations and ascertain whether there were pollution-generating activities in the microenvironment. Obtaining adequate time-activity information without burdening the subject becomes increasingly difficult as the number of days for which one needs exposure estimates increases.

Submodels are needed to determine microenvironmental concentrations under various conditions. Since most people spend 80 to 90 percent of their time indoors, it is particularly important to develop reliable submodels to account for penetration of outdoor pollutants indoors and contributions of indoor sources, such as smoking, cooking, vacuuming, etc., under various ventilation and source activity conditions. The submodels are often empirical (or statistical) models derived from indoor-outdoor measurements or semi-empirical models developed from a mass balance framework and with model parameters (loss rates and air exchange rates) derived from measurements. Many examples of these submodels exist for $PM_{2.5}$ and PM_{10} (see Lioy et al., 1990; Avol et al., 1996; Ozkaynak et al., 1996; Janssen, 1998). It is highly desirable to develop submodels that predict microenvironmental concentrations accurately for the specific subjects, homes, and schools of interest in the health study.

2.3.3 Reconciliation of Ambient and Personal Exposures

Several studies suggest that ambient concentrations are not highly correlated with either indoor concentrations or personal exposures to PM. For example, data from the P[illegible]M study in Riverside, California, indicated correlation (R) between ambient PM_{10} and perso[illegible] PM_{10}

accurately reflect the exposure of the human population (Ott et al., 1988; Spengler and Soczek, 1984; Brauer et al., 1989). Often exposures occurring indoors and/or in close proximity to outdoor sources are substantially different from that characterized by the nearest ambient air monitoring station.

2.3.1 Microenvironmental and Personal Measurement Studies

A large number of studies have examined indoor and outdoor differences in PM concentrations and attempted to quantify indoor source contributions to PM (Yocum et al., 1971; Lioy et al., 1985; Dockery and Spengler 1981; Spengler et al., 1981; Quackenboss et al. (1991); Sheldon et al., 1989; Leaderer et al., 1990). Most of these studies indicated that smoking was the single largest source of indoor particles. They also found that while there were significant differences in mass concentrations, there were striking similarities in indoor and outdoor sulfate levels in most homes. Similar studies of combustion products in homes have shown that unvented or otherwise improperly operated gas appliances are the main factor contributing to high indoor concentrations of NO_2 and CO. In the absence of indoor sources, infiltration of polluted outdoor air is confirmed to be the major factor explaining indoor levels.

Few studies have actually measured microenvironmental concentrations and personal exposures. For particles, the 1990 PTEAM study of 178 homes of non-smokers in Riverside, CA was a major advancement (Ozkaynak et al., 1995). The PTEAM study found that personal PM_{10} exposures were higher than those indicated by outdoor measurements. During the daytime, personal PM_{10} exposures were higher than indoor and outdoor concentrations measured with identical samplers. While personal activities and exposures (e.g., some smoking and cooking) were responsible for some of this difference, other factors were suspected of influencing the personal exposures. The motion of the individuals was suspected of enhancing the sampling rate compared to stationary indoor samplers. Also, the "personal cloud" of the subjects that included skin flakes, clothing lint, and resuspension of dust from nearby surfaces as they moved through rooms was suspected of enhancing the personal PM_{10} exposure levels. PTEAM also found that crustal elements (Si, Al, Fe, Cu) tended to be higher outdoors than indoors and were poorly correlated. The higher daytime crustal exposures suggested that people were being exposed to higher concentrations of larger particles outdoors and away from their home; conversely, they were being exposed to higher concentrations of smaller particles in their homes.

The PTEAM elemental sulfur concentrations were usually spatially uniform throughout the community and exhibited day-to-day changes with the meteorology. The correlation of indoor and outdoor elemental sulfur (which was mostly fine sulfate) was high ($r > 0.9$), suggesting the outdoor fine particle mass readily penetrates the indoor environment. The Koutrakis et al. (1992) and Leaderer et al. (1994) studies of New York homes reproduced most of the important findings from the PTEAM study.

exposures for nonsmoking adults was 0.37 for daytime samples, 0.54 for nighttime samples, and 0.42 for 24-hr samples. These cross-sectional correlations between personal and indoor concentrations were higher: 0.63, 0.88, and 0.74 for daytime, nighttime, and 24-hr averaged PM_{10} concentrations (Thomas et al., 1993). The Total Human Environmental Exposure Study (THEES) in New Jersey collected 24-hr PM_{10} samples for fourteen consecutive days and found a median individual correlation coefficient of 0.53 (range 0.14 to 0.90) for ambient and personal PM_{10} (Lioy et al., 1990). Incorporating time-activity data and comparing time-weighted indoor and outdoor concentrations with personal exposures produced slightly better correlation (median R=0.55). The Janssen et al. (1995) study in the Netherlands and the Bahadori et al. (1996) study in Nashville, Tennessee, have also shown moderate correlation for PM_{10} personal and ambient concentrations in cross-sectional analyses. This is an important issue for moderate and long-term air pollution health effects studies that rely on outdoor concentrations as the sole determinant of exposure or as an important input to determining the exposure of target populations. The lower than expected correlation may be due to (1) human PM exposures at work and/or in traffic that are only partially accounted for in indoor and outdoor measurements, (2) short-term exposures to high concentrations for short periods, such as from ETS, that are unaccounted for by the measurements, and (3) indoor and outdoor averages that reflect periods of low concentrations during which the subject may not be present.

The lack of correction often occurs because the between-subject variability is high. For example, Janssen et al (1995) studied smokers and nonsmokers and found large differences between individuals exposed and not exposed to ETS. This produces lots of scatter in the relationship and reduces correlation. Dramatic improvements in the relationship between ambient and personal PM were found when the data were analyzed longitudinally rather than cross-sectionally (Janssen, 1998). Other studies have confirmed that averaging over multiple days for each individual improves the strength of the association between personal and ambient PM_{10} concentrations. For example, even with the small number of consecutive samples collected in the PTEAM study, reanalysis of the data showed R increased from 0.42 to 0.57 with the simple time-averaging method (Ozkaynak and Spengler, 1996). Wallace (1996) found R increased from 0.24 to 0.68 when daily THEES data for all subjects for fourteen days were analyzed longitudinally instead of cross-sectionally.

Perhaps more important are findings that exposure models that account time-activity and other personal characteristics are able to improve characterizations of personal exposures far beyond what is achievable with ambient data alone. When daily time-activity data and personal characteristics from the THEES database were included (via stepwise regression) in a time-weighted exposure model (using indoor and outdoor concentrations), the correlation improved for all subjects to a median R of 0.93 (range 0.58 to 0.999) (Mage and Buckley 1995). Activities that are fairly easily captured in surveys and diaries, such as ETS exposure, cooking, use of unvented kerosene space heaters, and cooking, were important in the THEES PM_{10} exposure model. Thus, we expect that incorporating additional individual-level time-activity and housing characteristics data into exposure models for children in Fresno will produce significantly more accurate exposure assessment than would be possible using ambient concentrations alone.

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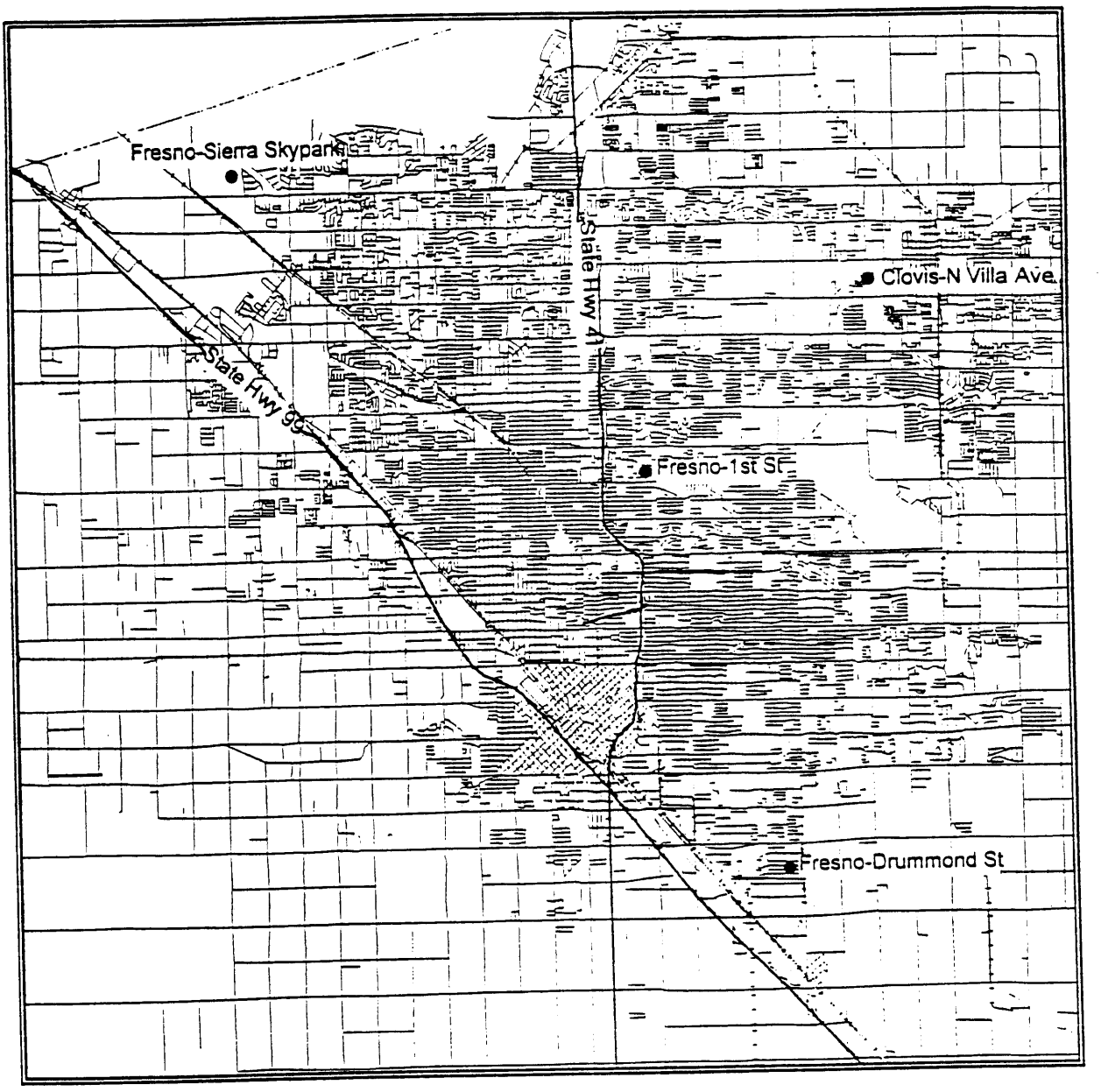

Figure 2-1. The roadways and routine air quality monitoring stations in the Fresno area.

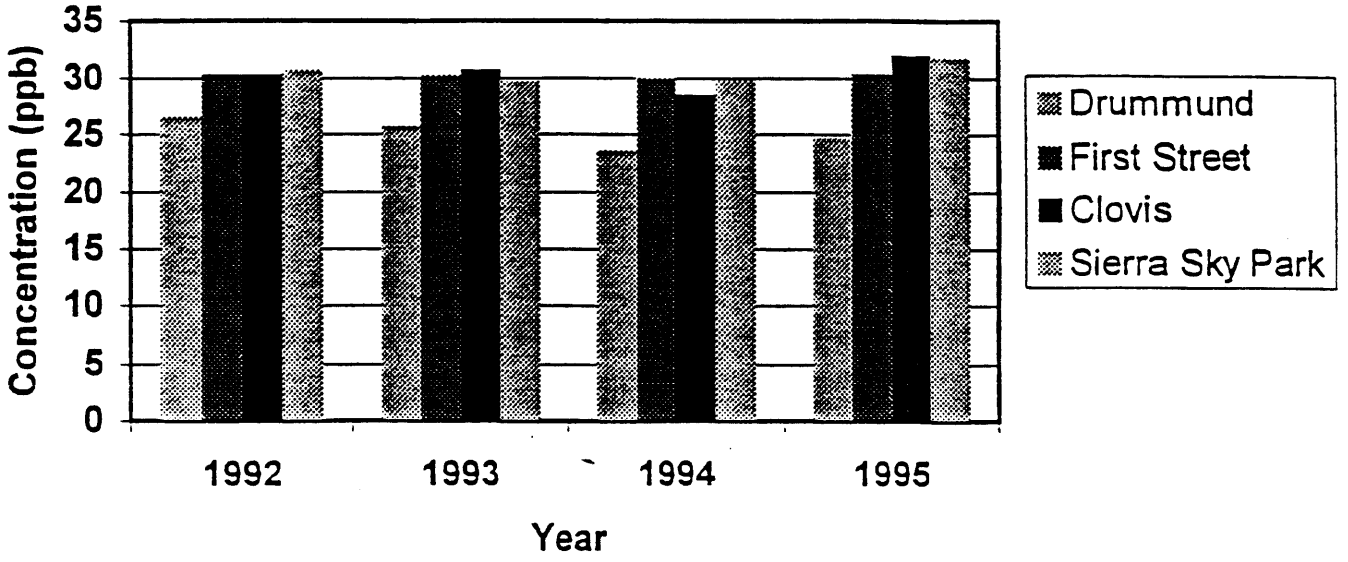

Figure 2-2. Annual average 24-hr ozone concentrations in Fresno and Clovis.

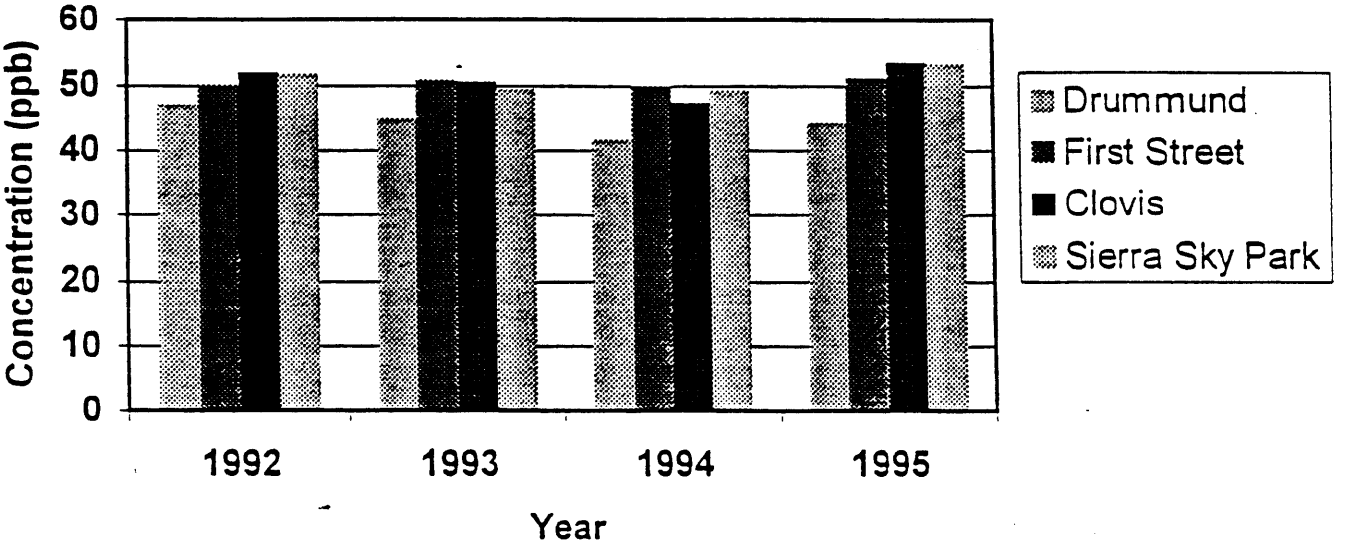

Figure 2-3. Annual average 10 AM – 6 PM ozone concentrations in Fresno and Clovis.

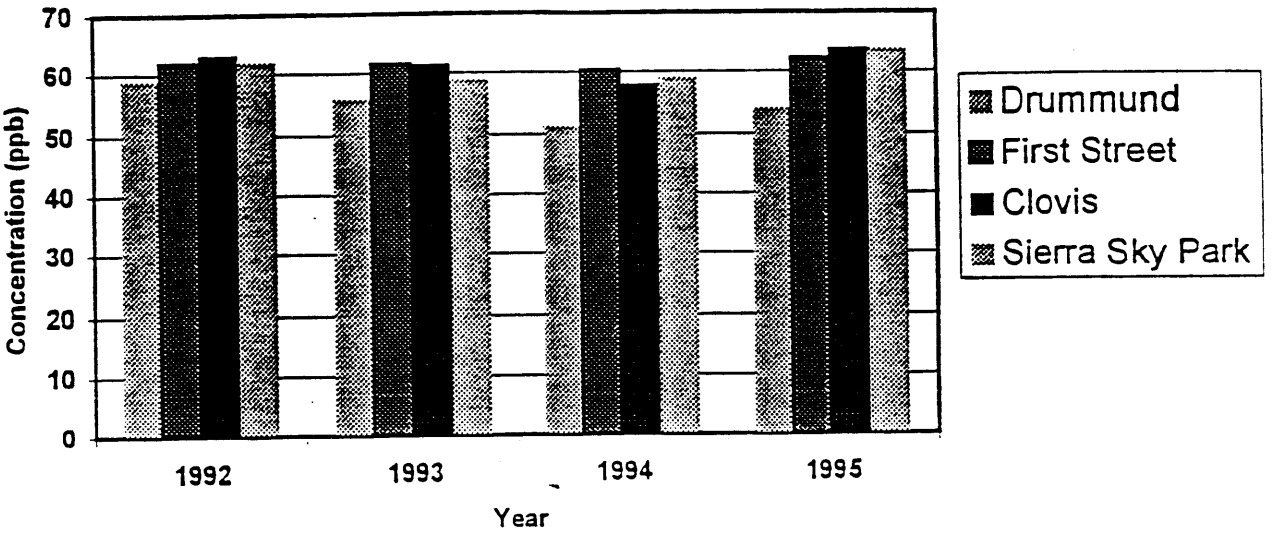

Figure 2-4. Annual average 1-hr daily maximum ozone in Fresno and Clovis.

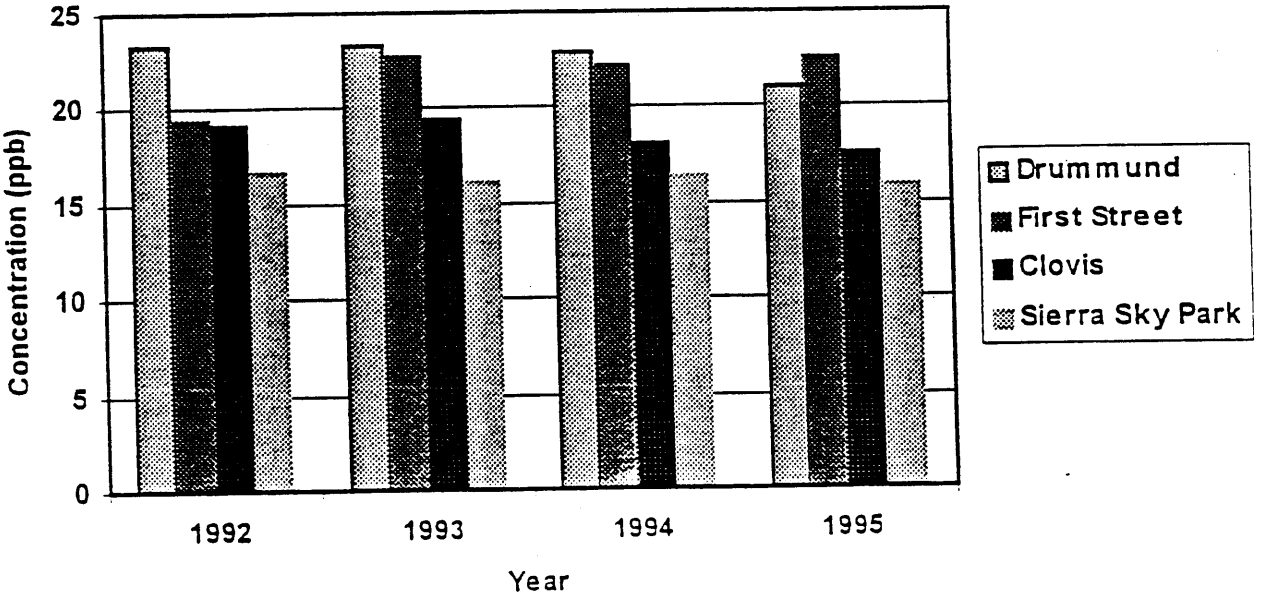

Figure 2-5. Annual average NO_2 concentrations in Fresno and Clovis.

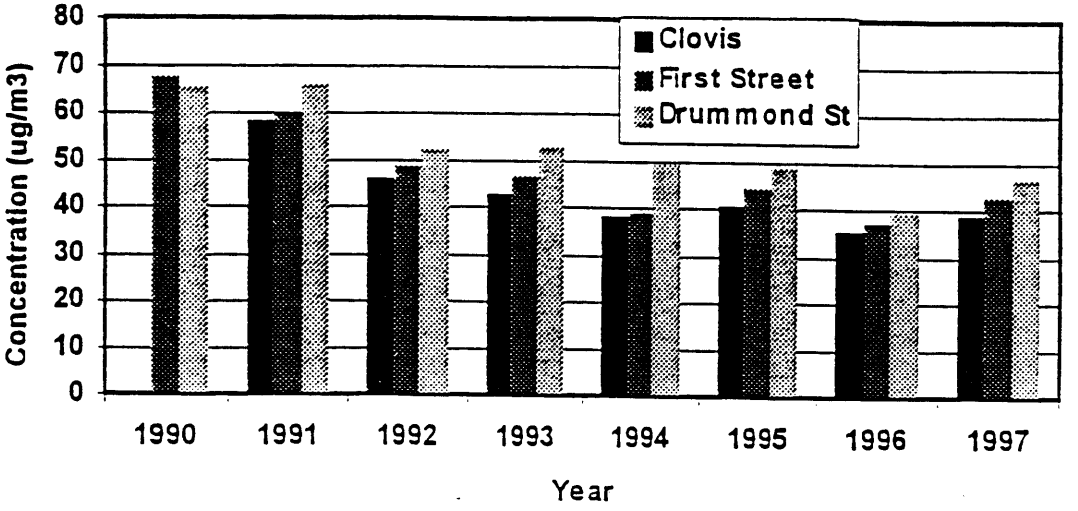

Figure 2-6. Annual average PM_{10} concentrations in Fresno and Clovis.

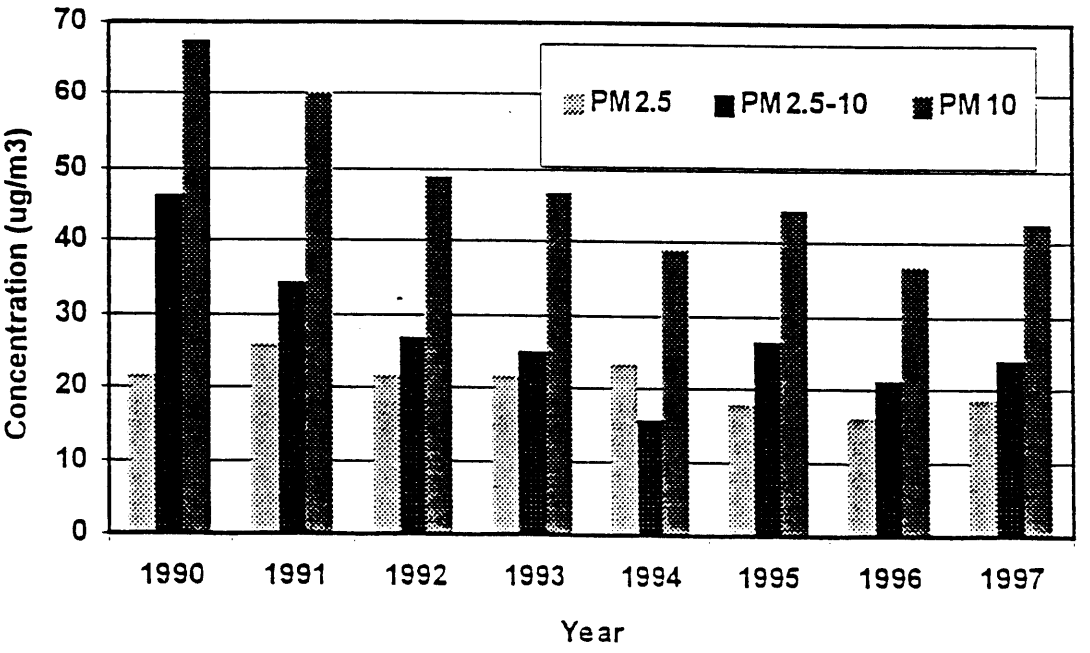

Figure 2-7. Annual average $PM_{2.5}$, $PM_{2.5-10}$, and PM_{10} at First Street in Fresno.

3. TECHNICAL APPROACH

3.1 OVERVIEW AND ASSUMPTIONS

The research proposed here, to characterize the air pollution exposure among asthmatic children in Fresno, California, is designed to harmonize with several other projects, with the ultimate goal of understanding the role of air pollution in the asthma experiences of these children. First, the study is fully integrated with the health aspect study proposed by Ira Tager, M.D., in Part A, which depends on the results of this work, Part B. Second, this exposure characterization is designed to build upon two intensive air quality measurement efforts to be conducted in the study region, the EPA "supersite" in Fresno and CRPAQS in the San Joaquin Valley, and to use the ARB mobile vans to assist in understanding neighborhood variations in air pollutant concentrations and the variation of concentrations inside and outside schools.

Ideally, the asthma study requires a knowledge of the daily exposures of each child to each agent of interest during each two week panel. A quick calculation reveals that this would involve over a million samples, which would cost several hundred million dollars to collect and analyze. Further calculations reveal that many of the resulting concentrations would be less than the limits of detection for most agents. Real-world knowledge tells us that children will not wear personal sampling gear for three two-week periods each year. Therefore, we propose to evaluate each child's exposure on each day of interest to each agent based on daily pollutant information from a central site, time-location data on each child, and models to be developed that relate concentrations at the central site to concentrations in important microenvironments, with factors included for neighborhood and home-specific parameters found to be important.

The basic hypothesis of this study is that the air pollutants of interest can be grouped into three categories on the basis of the principal determinants of their concentration.

- Regional pollutants, which will have similar concentrations throughout Fresno, so that measurements made at the central site will be similar to those made outside homes.
- Neighborhood pollutants, which will vary from the central site based on neighborhood characteristics, for instance, close proximity to a highway for those pollutants associated with traffic (e.g., elemental carbon).
- Home specific agents, which may have home sources, such as environmental tobacco smoke or cat dander.

To construct and refine these models we will examine the relationships between concentrations measured at the central site and those measured in four neighborhoods (during CRPAQS) and the relationships between concentrations measured at the central site and those measured outside and inside selected homes contemporaneously. Data on home-specific agents will be collected both with routine measurements of a few agents during the two-week health panels and with questionnaire and diary data on source usage (e.g., cigarette smoking and wood stove use).

The proposed research will:

- Add collection and analysis of key agents at the Fresno Supersite;
- Add collection and analysis of key agents at the four winter CRPAQS sites in Fresno neighborhoods;
- Conduct routine microenvironmental sampling for a few agents in all homes during the health panel studies;
- Conduct an intensive evaluation of the concentration of several agents inside and outside at a subset of homes ("exposure intensive");
- Evaluate traffic density in the subjects' neighborhoods;
- Conduct a small pilot study of personal exposures;
- Write and interpret questionnaire and diary data on source use;
- Write and interpret questionnaire and diary data on where subjects are at specified times;
- Develop field inspection sheets for homes and schools;
- Develop models to predict children's exposures;
- Evaluate each child's exposure to the relevant agents on each day of interest to the health study.

3.1.1 Agents of Interest

The agents of interest include both air pollutants and other agents which may confound or modify the effects of air pollution on asthma. The agents are listed below:

- Gases:
  - NO_2, NO
  - Ozone
  - SO_2
- Particles
  - $PM_{2.5}$
    - Mass
    - Organic carbon/elemental carbon (OC/EC)
    - Sulfate ion
    - Nitrate ion
    - Ammonium ion
    - Metals
    - Potassium
    - PAHs (polycyclic aromatic hydrocarbons)
    - Particle number
  - PM_{10}
    - Mass
    - Metals
- Environmental tobacco smoke (ETS)
- Biological agents
  - Pollens

- Fungal spores
- Endotoxin
- Allergens in house dust
  - Dust mites
  - Cat dander
  - Dog dander
  - Cockroach eggs

Table 3-1 describes where each of these agents will be monitored (central site, neighborhood sites, subjects' homes, personal samples, schools) and the sample duration. An additional agent which had originally had been considered is latex particles, but the results of a previous ARB/Cal Tech study by Ann Miguel led us to believe performing these expensive analyses would not yield useful data. The researchers in that study did not find detectable levels of latex allergen in any of the samples of paved road dust collected from the middle two-thirds of city surface streets in Los Angeles; the only location where detectable levels were found was particles deposited on a guardrail at a freeway interchange.

The relationship between asthma and air pollution cannot be understood without an evaluation of exposures to potential confounders and effect modifiers. Valid interpretation of any associations between various components of PM and/or gaseous ambient pollutants and asthma symptoms or changes in lung function is critically dependent upon the control of a number of environmental confounders and effect modifiers. These have been discussed in detail in Part A (Section 2, "Background"). Perhaps the most important potential confounder/effect-modifier in the age group under study is exposure to environmental tobacco smoke (ETS) in the home. Tobacco smoking in the home is the most important indoor source for the generation of PM. In addition, exposure to ETS is associated with worsening symptoms of asthma and lowered levels of lung function. Day-to-day differences in exposure may also modify (enhance) any effects of exposure to ambient PM or gaseous pollutants. Seasonal variation in plant pollens and day-to-day variation within pollen seasons may also confound or modify any association between asthma symptoms/lung function and ambient pollutants. Exposure to household allergens such as dust mites, cockroaches, cat dander, etc. may have a seasonal component that can modify asthmatic responses to air pollution. It will be important in the early panels to determine if these exposures are relatively stable from day to day and to define the seasonal component (e.g., mites generally do not survive in dry conditions). Finally, there is considerable interest in the potential role of endotoxin both in the home and in the ambient environment on symptom severity and lung function in asthmatic children. Virtually no data are available on the day-to-day and seasonal variations in such exposure, and data will need to be collected to determine if endotoxin either confounds or modifies responses to ambient air pollutants.

The exposure protocols are designed to provide data on all of these exposures. Few, if any previous studies of air pollution health effects have tried to account for the effects of all of these important environmental confounders/modifiers.

Table 3-1. Proposed agents to be sampled and sample duration at various locations.

Location	Central Site		Two-Week Panel		Exposure Intensive[3]		Personal	Schools
	Fresno Supersite (on-going)	4 neighborhood sites during CRPAQS Intensive	Inside Home	Inside & outside Home	Inside Home	Outside home	Selected students	Selected sites w/van
Year(s) of study	1-5	1	1-2	1-3	2	2	2	2,3
No. of years	5	15 days in 2 months	2	5 summer months	1	1		
Agent:								
NO_2	H[1]	H[1]	MD		MD[4]	MD	MD	H[5]
NO	H[1]	H[1]						H[5]
SO_2	H[1]							H[5]
Ozone	H[1]	H[1]		MD	MD[4]	MD[4]	MD	H[5]
ETS			MD		MD		MD	
House dust allergens & endotoxins			G					
Spores & pollen	D[2]	D			MD	MD		D[5]
PM_{10} endotoxins	D[2]	D			MD	MD	MD	D[5]
Particle scattering	H[1]	H[1]			H	H		H[5]
$PM_{2.5}$ mass	H[1]	D[1]			MD	MD	MD	H[5]
$PM_{2.5}$ ions	D[1]	D[1]			MD	MD		H[6]
$PM_{2.5}$ metals	D[1]	D[1]						D[5]
$PM_{2.5}$ OC/EC	H[1]	D[1]			MD	MD		H[5]
PM_{10} mass	H[1], D[5] (1 in 6)	D[1] (1 in 6)			MD	MD	MD	H[5]
PM_{10} metals	D[5] (1 in 6)				2W	2W		

Sample duration: H = hour, D = 1 day (24 hour), MD = multi-day, W = weekly, G = grab, 2W = 2-week

[1] Operations & data to be provided by CRPAQS (DRI/ARB/EPA)

[2] Samples collected years 1-5; only analyzed years 1-2

[3] All MD and 2W samples collected Tuesday 4pm to Friday 8am (excluding 8am to 4pm daily), Friday 4pm to Monday 8am, and Monday 4pm to Thursday 8am (excluding 8am to 4pm daily); 3 MD samples analyzed separately, 2W samples collected on 1 filter and analyzed together.

[4] As part of two-week panel measurments

[5] Operations & data to be provided by ARB

3.1.2 Major Components of the Measurement Phase of the Exposure Assessment Study

The components of the exposure assessment study, in which measurements of these agents will occur, are as follows:

- The central site (Fresno First Street), where concentrations of air pollutants will be collected hourly or daily for the full five years of the study
- The four neighborhood sites in Fresno, where concentrations of air pollutants will be collected hourly or daily in December 2000 and January 2001 as part of the CRPAQS Winter Intensive Study
- The Routine Microenvironmental Measurements which will be made in the homes of all subjects during all two-week panels for the first two years of the study
- The Exposure Intensive component, in which detailed measurements will be made inside and outside of the homes of a subset of the subjects during one of the two-week health panels
- The personal sampling, which is a pilot study of a few children for selected air pollutants, and will be performed in conjunction with the exposure intensive component
- The school component, in which measurements will be made with the mobile van indoors and outdoors at selected schools

Table 3-2 outlines the types and number of samples which will be collected in each of these phases of the study.

3.1.3 Data Analysis and Modeling Phase

Questionnaires and diaries will be developed to document housing characteristics and potential pollution sources that are home specific, e.g., cigarette smoking and use of fireplaces and wood stoves. Other diaries will record the child's location at selected times of day during the two week health panels. Neighborhoods with participant's homes will be classified based on estimates of traffic density (from CALTRANS) and vegetation types and biomass (from biogenic emission databases. Extensive statistical analysis of the central site data, supplemental CRPAQS data, routine monitoring data, home exposure intensive data, ARB van data, and questionnaire data will be conducted. Subsequently, models will be constructed to relate concentrations measured at the central site to those measured in neighborhoods and outside and inside homes. Additional models will be developed to estimate children's exposures based on their location at various points in time and estimations of micorenvironmental concentrations.

3.1.4 Key Resource Assumptions

This proposal relies upon the continued operation of the central site in Fresno throughout the five years of the health study, and the availability of high quality data from that study. This proposal also relies on the operation of the four neighborhood sampling sites for CRPAQS and availability of high quality data from that study. This proposal also assumes the availability of the mobile vans operated by ARB personnel and equipped with continuous monitors for gaseous and particle matter agents as outlined in Section 4.5, and that ARB would perform the

Table 3-2. Proposed agents to be sampled and sample numbers at various locations.

Location	Central Site		Two-Week Panel		Exposure Intensive[3]		Personal	Schools
	Fresno Supersite (on-going)	4 neighborhood sites during CRPAQS Intensive	Inside Home	Inside & outside Home	Inside Home	Outside home	Selected students	Selected sites w/van
Year(s) of study	1-5	1	1-2	1-3	2	2	2	2,3
No. of years	5 (during panels)	15 days in 2 months	2	5 summer months	1	1		
Agent:								
NO_2	H[1]	H[1]	1760		MD[4]	165	25	H[5]
NO	H[1]	H[1]						H[5]
SO_2	H[1]							H[5]
Ozone	H[1]	H[1]		1980	MD[4]	MD[4]	25	H[5]
ETS			1227		248		25	
House dust allergens & endotoxins			1665					
Spores & pollen	493	66			495	495		D[5]
PM_{10} endotoxins	493	66			165	165	25	D[5]
Particle scattering	H[1]	H[1]			H	H		H[5]
$PM_{2.5}$ mass	H[1]	D[1]			495	495	25	H[5]
$PM_{2.5}$ ions	D[1]	D[1]			165	165		H[5]
$PM_{2.5}$ metals	D[1]	D[1]						D[5]
$PM_{2.5}$ OC/EC	H[1]	D[1]			495	495		H[5]
PM_{10} mass	H[1], D[5] (1 in 6)	D[1] (1 in 6)			495	495	25	H[5]
PM_{10} metals	D[5] (1 in 6)				165	165		

Sample duration: H = hour, D = 1 day (24 hour), MD = multi-day, W = weekly, G = grab, 2W = 2-week

[1] Operations & data to be provided by CRPAQS (DRI/ARB/EPA)

[2] Samples collected years 1-5; only analyzed years 1-2

[3] All MD and 2W samples collected Tuesday 4pm to Friday 8am (excluding 8am to 4pm daily), Friday 4pm to Monday 8am, and Monday 4pm to Thursday 8am (excluding 8am to 4pm daily); 3 MD samples analyzed separately, 2W samples collected on 1 filter and analyzed together.

[4] As part of two-week panel measurements

[5] Operations & data to be provided by ARB

necessary laboratory analyses and quality control for the ARB sampling van data. Furthermore, we will rely on personnel from the health team to distribute or administer the necessary questionnaires and diaries, to place and retrieve the samplers for the routine home microenvironmental monitoring, and to ship these samples to the laboratories for analysis.

Specifically, our key assumptions regarding the health team field staff are as follows:

- They will deploy and collect passive monitors during the two-week panels. They will collect the house-dust samples (using a special vacuum cleaner we will supply) during the home visits during each two-week panel.
- They will handle the collected samples and ship them to the appropriate laboratories.
- They will provide us documentation of their activities in our behalf.
- They will coordinate with us regarding home selection and visit scheduling. They will be the primary contact with children and families involved in the study, and will make the first contact with them.
- They will enter time-activity data and house-survey data into a database for use by both the health and exposure teams.

Our principal assumptions about field and data support related to the Fresno central site (First Street) and the four CRPAQS winter neighborhood sites are that we will be provided:

- Routine data from the ARB and the San Joaquin Valley Unified Air Quality Management District.
- Space at the Fresno Supersite to set up sampling equipment, reasonable access to the roof, etc. for sampling inlets and installation of the outdoor samplers. Sufficient electrical power to operate the equipment, and access to the site at any time to fix, repair, and operate equipment.
- Similar access to and support at the four CRPAQS sites to be used for enhanced monitoring and sample collection during the CRPAQS Winter Intensive Study.
- Continued operation of and validation of data from the ARB Fresno-First Street site as part of CRPAQS and the EPA-sponsored PM Supersite network. These measurements include hourly (or sub-hourly) continuous monitoring for many gaseous and particulate-matter agents, plus some integrated sample collection for later analysis in the laboratory (--see the first two columns in Table 3-1).
- Support from the Fresno Supersite operator for filter changing and shipping.
- Operation of and validation of data from four neighborhood sites in the Fresno area during the CRPAQS two-month winter intensive-sampling program during December 2000 and January 2001. These measurements will include hourly (or sub-hourly) continuous monitoring for many gaseous and particulate-matter agents, plus daily integrated sample collection for later analysis in the laboratory (the concentrations of ozone, nitrogen oxides, particle scattering, $PM_{2.5}$ mass, PM_{10} mass, $PM_{2.5}$ sulfate, $PM_{2.5}$ nitrate, $PM_{2.5}$ ammonium ions, $PM_{2.5}$ metals, $PM_{2.5}$ organic carbon, $PM_{2.5}$ elemental

carbon, PM_{10} metals--see the first and third columns in Table 3-1 for those measurements of interest for this study).

Our principal assumptions about field and data support related to the ARB mobile van are that:

- A mobile van will be equipped for air pollution measurements and operated by ARB staff.
- Access to the mobile van for our use in neighborhoods and schools during the second and third years of the study.
- ARB will perform the necessary equipment calibration and laboratory analyses of samples collected by the mobile van.
- ARB will provide quality-control data to us in a standard format and in a timely fashion.

We also assume that the ARB will support us in the following ways:

- Support our efforts to piggy-back with existing routine and special study monitoring programs.
- Help us obtain limited auditing support from ARB to support internal project QA/QC efforts. This may include sample exchange with ARB or local district laboratories.
- Provide routine monitoring data from the ARB network for use in the QA/QC effort.

3.2 AGENTS AND METHODS

3.2.1 Overview of Sampling Methods

Sampling methods will vary with the location of the samplers. Those samples routinely collected at the central site and as part of the CRPAQS winter intensive study will not be described here. To those sites we will add Burkhard samplers for pollen and fungal spores, and a sampler for endotoxin (see below, under bioaerosol sampling). At the homes during the routine microenvironmental sampling, passive monitoring will be relied upon for the collection of nitrogen dioxide, ozone, and environmental tobacco smoke samples. House dust will be collected with a special vacuum cleaner adapted for this purpose. During the exposure intensive study active sampling will be used in a subset of subjects' homes to collect particles for compositional analysis for mass, metals, potassium, ions, and endotoxin. These samples will be collected during three time periods:

1. Tuesday 4 pm to Friday 8 am (excluding 8 am to 4 pm daily)
2. Friday 4 pm to Monday 8 am, and
3. Monday 4 pm to Thursday 8 am (excluding 8 am to 4 pm daily)

The multi-legged filter sampler for the exposure intensive measurements indoors and outdoors will be similar in concept to the two-week sampler designed and used in the ARB-sponsored Children's Health Study in Southern California (Main et al., 1994). The sampler will include the following legs using a timer to operate on the schedule listed above:

- A $PM_{2.5}$ leg with a Teflon filter followed by a Nylon backup filter for mass, sulfate, nitrate, and ammonium ion.
- A second $PM_{2.5}$ leg with a non-greased inlet and a Quartz filter for OC/EC followed by a filter for ETS.
- A PM_{10} leg with a Teflon filter for mass and metals.

The sampler would also contain a separate PM_{10} leg with a separate timer to collect filters for endotoxins. Small, quiet 4 lpm pumps will be used in this sampler. For use indoors, the sampler will be mounted on a cart much like a mail-distribution cart with wheel locks. The filter legs will hang upside down from the top of the cart. For use outdoors, we will use a similar setup, but with a simple cover for protection from the weather.

Nitrogen Dioxide

Nitrogen dioxide samples will be collected in the homes during the routine microenvironmental sampling and the exposure intensive sampling studies with a passive sampler. The sampler consists of a small acrylic tube with three triethanolamine-coated screens inserted at one end. Nitrogen dioxide molecules diffuse to the screens and react with the triethanolamine. The screens are then desorbed and analyzed spectrophotometrically. The samplers have been used in nitrogen dioxide studies for over 20 years, and are widely accepted as a reliable method well suited to environmental measurements. The samplers will be provided by and analyzed by Research Triangle Institute (RTI) of North Carolina.

Ozone

Ozone measured at the home will be measured by passive samplers operating for two weeks during the summer (May through September) season, when ozone is at relatively high levels. Ozone will be collected on the Ogawa samplers, which depends on passive diffusion of the ozone molecules to the collection medium, where ozone oxidizes nitrite to nitrate. The nitrate is then desorbed from the sampler and measured by ion chromatography with conductivity detection. The samplers will be provided by and analyzed by RTI of North Carolina. Because of limit of detection problems and problems at low ozone levels, we have chosen to sample only for two-week periods, and not try to collect the two- to four-day samples like the other analytes.

Substrate Preparation

The choice of filter type results from a compromise among the following filter attributes: 1) mechanical stability; 2) chemical stability; 3) particle or gas sampling efficiency; 4) flow resistance; 5) loading capacity; 6) blank values; 7) artifact formation; 8) compatibility with analysis method; and 9) cost and availability. U.S. EPA filter requirements for $PM_{2.5}$ sampling specify 0.3 μm DOP (dioctylphthalate) sampling efficiency in excess of 99.7%, weight losses or gains due to mechanical or chemical instability of less than 20 μg/filter equivalent, and alkalinity of less than 25 microequivalents/g to minimize sulfur dioxide (SO_2) and nitrogen oxides (NO_x) absorption (Federal Register, 1997).

The substrates to be used for this study are as follows: 1) Gelman (Ann Arbor, MI) polymethylpentane ringed, 2.0 mm pore size, 47-mm diameter PTFE Teflon-membrane filters (#R2PJ047) for particle mass, ions, and elements; and 2) Pallflex (Puttnam, CT) 47-mm diameter pre-fired quartz-fiber filters (#2500 QAT-UP) for organic and elemental carbon measurements.

The manufacturer's identification numbers are important specifications since these filters have been found to meet the requirements for aerosol sampling. Watson et al. (1988a; 1988b) address concerns about artifact formation and contamination of these filter media and demonstrate that these substrates are the most appropriate for the prescribed measurements. Filters are 47-mm diameter disks which are compatible with the Nuclepore filter holders used in the sequential filter sampling system.

These filter substrates require treatment and representative chemical analyses before they can be used (Chow, 1987). Discoveries of excessive blank levels and filter interferences in several previous monitoring programs which neglected these acceptance tests have compromised the results of those studies. At least two filters from each set of 100 purchased from the specified manufacturers is analyzed for the relevant species to verify that pre-established specifications have been met. Each filter is individually examined prior to labeling for discoloration, pinholes, creases, or other defects.

Lots are rejected if they do not pass these acceptance tests. Testing of sample media continues throughout the project. Ten percent of the samples are designated as laboratory and field blanks, and these follow the handling procedures except for actual sampling. Sample pre-treatment for this study includes the following:

- Pre-firing of Quartz-Fiber Filters. Quartz-fiber filters absorb organic vapors over time. Blank quartz-fiber filters are heated for at least three hours at 900 °C. Two samples of each batch of 100 pre-fired filters are tested for carbon blank levels prior to sampling, and sets of filters with carbon levels exceeding 1 $\mu g/cm^2$ are re-fired or rejected. Pre-fired filters are stored in a freezer prior to preparation for field sampling.

- Equilibrating Teflon-Membrane Filters. On several occasions, batches of Gelman ringed Teflon filters have yielded variable (by up to 100 μg/filter over a few days) blank masses. As the time from manufacture increases, this variability decreases. Since Gelman has minimized its long-term inventory of these filters, and is manufacturing them on an as-

ordered basis, this variability is being observed with greater frequency. A one-month conditioning period of separated filters in a controlled environment followed by one week of equilibration in the weighing environment (e.g., ±2°C temperature between 20°C and 23°C, and ±5% relative humidity between 30% and 40%) is currently being applied to these filters, and this appears to have reduced the variability to acceptable (within ± 15 µg/filter for re-weights of 47 mm or 37 mm diameter filters) levels. Sets of Teflon-membrane filters which exceed twice XRF detection limits for elements are rejected.

The results of the filter treatments, chemical analyses, and visual inspections are recorded in a database with the lot numbers. A set of filter IDs is assigned to each lot so that a record of acceptance testing can be associated with each sample.

Mass of Particles

The mass of particles will be determined gravimetrically. Filters will be pre-weighed and post-weighed on a microelectrobalance in a temperature and humidity controlled room. Prior to weighing, filters will be placed in the room for 24 hours to equilibrate. The charge on each filter is neutralized by a polonium^{210} radiation source for 30 seconds prior to being placed on the balance pan. The room will be kept at 72 $\pm$ 3° F and a constant relative humidity. The temperature and humidity will be recorded when the filters are placed in the weighing room, and at the beginning and end of the weighing session, and at least every 2 hours during weighing. A strict weighing protocol will be written and followed, and will include quality assurance measures similar to the following, to be performed on each day that weighing takes place:

- initial calibration of the balance with standard weights
- zeroing of the balance
- rechecking the calibration weight, which must be within a written tolerance, or these first three steps are repeated until they are
- weighing a quality assurance filter of the same type as the field samples, but which is kept in the weighing room at all times; if this is within written tolerances, proceed to weigh filters
- after each 10 filters are weighed, the balance is re-zeroed
- after each 20 filters are weighed, the quality assurance filter is weighed again, and the balance is re-zeroed; if the quality assurance filter is outside of the acceptable range, the 10 sample filters are reweighed after the balance is recalibrated and re-zeroed as above.

Sulfate, nitrate, and ammonium ions

Ion chromatography is widely accepted as the preferred method for determining the concentration of anions and cations in solution. Charles Perrino will be performing the analysis at the Environmental Health Sciences Lab, at the University of California, Berkeley. His experience in the technique includes analysis of environmental samples at a groundwater-contaminated site at Sandia National Laboratory in Livermore, CA. The analysis involved

7 anions and 8 cations, including sulfate, nitrate, and ammonium ions. The severe matrix effects of both sodium and chloride ions in the Livermore groundwater were accounted for using a variety of filters and chemical suppression. This worst-case scenario will be good experience for personnel working with any samples for the Fresno project. Detection limits for anions and cations will be determined using spiked samples. Acceptable recoveries of spiked samples will also be determined once any interferences from the sample matrix are identified. Sulfate and nitrate anions will be characterized by collecting samples and analyzing them using ion chromatography (IC) consistent with the standard EPA method. Samples will be dissolved in deionized water and filtered using a C18 sep-pak cartridge and a 0.45 μm aqueous-compatible disk. An aliquot of each sample will be injected onto an IC-Pak anion column equipped with a chemical suppressor. The eluent used will be 1.6mM $NaHCO_3$ / 1.4 mM Na_2CO_3. Standards (0.1 μg/ml through 100 μg/ml) will be run on each analysis day. Ammonium cations will be characterized by collecting samples and analyzing them using IC as well. Sample preparation will be similar to the anion analysis. Samples will be dissolved in DI water and filtered using a C18 sep-pak cartridge and a 0.45 um aqueous-compatible disk. An aliquot of each sample will be injected onto an IC-Pak cation column equipped with a chemical suppressor. The eluent used will be 3mM HNO_3 / 0.1 mM EDTA. Standards (0.05 μg/ml through 20 μg/ml) will be analyzed on each analysis day.

Metals and Potassium

Metals and potassium will be measured on collected particles by X-ray Diffraction. These analyses will be performed by Desert Research Institute (DRI). X-ray fluorescence (XRF) analysis is performed on Teflon-membrane filters for 40 elements (Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Y, Zr, Mo, Pd, Ag, Cd, In, Sn, Sb, Ba, La, Au, Hg, Tl, Pb, and U) with an energy dispersive x-ray fluorescence (EDXRF) analyzer (Watson et al., 1999).

In XRF, inner shell electrons are removed from the atoms of the aerosol deposit. An x-ray photon with a wavelength characteristic of each element is emitted when an outer shell electron occupies the vacant inner shell. The number of these photons is proportional to the number of atoms present. The characteristic x-ray peaks for each element are defined by 200 keV-wide windows in an energy spectrum ranging from 1 to 50 keV.

XRF analyses are performed on a Kevex Corporation Model 700/Delta energy dispersive x-ray fluorescence (EDXRF) analyzer using a side-window, liquid- cooled, 60 keV, 3.3 milliamp rhodium anode x-ray tube and secondary fluorescers. The x-ray output stability is within 0.25% for any 8-hour period within a 24-hour duration. The silicon detector has an active area of 30 mm^2, with system resolution better than 165 eV. The analysis is controlled, spectra are acquired, and elemental concentrations are calculated by software implemented on an LSI 11/23 microcomputer which is interfaced to the analyzer.

Three types of XRF standards are used for calibration, performance testing, and auditing: 1) vacuum-deposited thin-film elements and compounds (Micromatter); 2) polymer films (Dzubay et al., 1981); and 3) NIST (National Institute of Standards and Technology, formerly National Bureau of Standards) thin-glass films. The vacuum deposits cover the largest number of elements and are used as calibration standards. The polymer film and NBS standards are used

as quality control standards. NIST standards are the definitive standard reference material, but these are only available for the species Al, Ca, Co, Cu, Mn, and Si (SRM 1832) and Fe, Pb, K, Si, Ti, and Zn (SRM 1833). A separate Micromatter thin-film standard is used to calibrate the system for each element.

Sensitivity factors (number of x-ray counts/$\mu g/cm^2$ of the element) are determined for each excitation condition. These factors are then adjusted for absorption of the incident and emitted radiation in the thin film. These sensitivity factors are plotted as a function of atomic number and a smooth curve is fitted to the experimental values. The calibration sensitivities are then read from these curves for the atomic numbers of each element in each excitation condition. The polymer film and NIST standards are analyzed on a periodic basis using these sensitivity factors to verify the calibration accuracy. The stability of the instrument response is monitored by analyzing a multilayer Micromatter thin-film standard with each run of 15 filters. When deviations from specified values are greater than ± 5%, the system is re-calibrated.

The sensitivity factors are multiplied by the net peak intensities yielded by ambient samples to obtain the $\mu g/cm^2$ deposit for each element. The net peak intensity is obtained by the following: 1) subtracting background radiation; 2) subtracting spectral interferences; and 3) adjusting for x-ray absorption.

During XRF analysis, filters are removed from their Petri slides and placed with their deposit sides downward into polycarbonate filter cassettes. A polycarbonate retainer ring keeps the filter flat against the bottom of the cassette. These cassettes are loaded into a carousel in the x-ray chamber that contains 16 openings. The filter IDs are recorded on a data sheet to correspond to numbered positions in the carousel. The sample chamber is evacuated to 10^{-3} torr and a computer program controls the positioning of the samples and the excitation conditions. Complete analysis of 16 samples under five excitation conditions requires approximately seven to eight hours. The vacuum in the x-ray chamber and the heat induced by the absorption of x-rays can cause certain materials to volatilize. For this reason, labile species such as nitrate and organic carbon are measured on a quartz-fiber filter, rather than on the Teflon-membrane filter which is subjected to XRF analysis.

A quality control standard and a replicate from a previous batch are analyzed with each set of 14 samples. When a quality control value differs from specifications by more than ± 5% or when a replicate concentration differs from the original value (when values exceed 10 times detection limits) by more than ± 10%, the samples are re-analyzed. If further tests of standards show that the system calibration has changed by more than ± 3%, the instrument is re-calibrated as described above.

Organic and Elemental Carbon

Particles will be collected on special filters prepared in advance by DRI. They will then be analyzed by DRI for organic carbon and elemental carbon using the thermal optical transmission method.

The thermal/optical reflectance (TOR) method measures organic and elemental carbon. The TOR method is based on the principle that different types of carbon-containing particles are

converted to gases under different temperature and oxidation conditions (Chow et al. 1993). The different carbon fractions from TOR are useful for comparison with other methods which are specific to a single definition for organic and elemental carbon. These carbon fractions also distinguish among seven carbon fractions reported by TOR: 1) the carbon evolved in a helium atmosphere at temperatures between ambient and 120°C (OC1); 2) the carbon evolved in a helium atmosphere at temperatures between 120°C and 250°C (OC2); 3) the carbon evolved in a helium atmosphere at temperatures between 250°C and 450°C (OC3); 4) the carbon evolved in a helium atmosphere between 450°C and 550°C (OC4); 5) the carbon evolved in an oxidizing atmosphere at 550°C (EC1); 6) the carbon evolved in an oxidizing atmosphere between 550°C and 700°C (EC2); and 7) the carbon evolved in an oxidizing atmosphere between 700°C and 800°C (EC3).

The thermal/optical reflectance carbon analyzer consists of a thermal system and an optical system. The thermal system consists of a quartz tube placed inside a coiled heater. The current through the heater is controlled to attain and maintain pre-set temperatures for given time periods. A portion of a quartz filter is placed in the heating zone and heated to different temperatures under non-oxidizing and oxidizing atmospheres. The optical system consists of a He-Ne laser, a fiber optic transmitter and receiver, and a photocell. The filter deposit faces a quartz light tube so that the intensity of the reflected laser beam can be monitored throughout the analysis.

As the temperature increases from ambient (~25°C) to 550°C, organic compounds are volatilized from the filter in a non-oxidizing (He) atmosphere while elemental carbon is not oxidized. When oxygen is added to the helium at temperatures greater than 550°C, the elemental carbon burns and enters the sample stream. The evolved gases pass through an oxidizing bed of heated manganese dioxide where they are oxidized to carbon dioxide, then across a heated nickel catalyst which reduces the carbon dioxide to methane (CH_4). The methane is then quantified with a flame ionization detector (FID).

The reflected laser light is continuously monitored throughout the analysis cycle. The negative change in reflectance is proportional to the degree of pyrolytic conversion from organic to elemental carbon that takes place during organic carbon analysis. After oxygen is introduced, the reflectance increases rapidly as the light-absorbing carbon is burned off the filter. The carbon measured after the reflectance attains the value that it had at the beginning of the analysis cycle is classified as elemental carbon. This adjustment for pyrolysis in the analysis is significant, as high as 25% of organic or elemental carbon, and it cannot be ignored. Johnson et al. (1981) reported that an average of 22% of the organic carbon in the samples they analyzed was pyrolytically converted to elemental carbon as evidenced by reflectance. The precision of the pyrolytic conversion has been found to be ± 10% in both organic and elemental carbon (Johnson et al., 1981).

A 0.5 cm^2 circular punch is removed from a quartz-fiber filter and this punch is placed vertically into a quartz boat which is inserted into the oven area with a thermocouple pushrod. The atmosphere flowing through the oven at this stage is pure helium. The temperature ramps from ~25°C to 120°C, to 250°C, to 450°C, to 550°C and the FID output is recorded every two seconds on a microcomputer data acquisition system. A 2% oxygen (O_2) in helium (He) atmosphere is introduced at 550°C, followed by temperature increases to 700°C, then to 800°C. The microcomputer controls the time intervals, monitors the temperatures, FID output, and

reflectance. The FID response is integrated over the pre-specified temperature, oxidation, and reflectance intervals. The fractions in the intervals corresponding to reflectances less than or equal to the initial value are summed to yield organic carbon, and fractions in the intervals with reflectances greater than the initial value in the oxidizing atmosphere are summed to yield elemental carbon.

For aluminum substrates from MOUDI samplers, the process is similar, except that the quartz light tube is removed and the reflected laser beam is not used to monitor reflectance. Organic carbon is defined as the sum of the first four peaks plus the pyrolysis correction determined from the analysis of the MOUDI after-filter. Elemental carbon is defined as the sum of the last three peaks minus the pyrolysis correction.

The system is calibrated by analyzing samples of known amounts of methane, carbon dioxide, and potassium hydrogen phthalate (KHP). The FID response is rationed to a reference level of methane injected at the end of each sample analysis. Performance tests of instrument calibration are conducted at the beginning and end of each day's operation. Intervening samples are re-analyzed when calibration changes of more than ± 10% are found.

Known amounts of American Chemical Society (ACS) certified reagent grade crystal sucrose and KHP are committed to TOR as a verification of the organic carbon fractions. Fifteen different standards are used for each calibration. Widely accepted primary standards for elemental and/or organic carbon are still lacking.

Environmental Tobacco Smoke

Environmental tobacco smoke (ETS) will be sampled by collecting nicotine as a tracer. Nicotine is collected by passive diffusion to a filter treated with sodium bisulfate, with which the nicotine reacts. The sampler itself consists of a modified industrial hygiene sampling cassette with an inert filter as a windscreen. The sampler is a disk approximately 1.5 inches in diameter and 1 inch high, made of plastic. It weighs half an ounce. The nicotine is extracted from the filter in ethanolic water, the pH is adjusted with sodium bisulfate to release the free nicotine molecule, which is then concentrated by liquid liquid extraction into heptane. A small aliquot of the heptane layer is then injected into a gas chromatograph with a nitrogen selective detector. Standards (0.02 μg/ml through 2 μg/ml) are run on each analysis day, as are a solvent blank, a blank filter which has not left the laboratory, and at least three filters spiked with known amounts of nicotine. Recovery must average at least 95% with a coefficient of variation less than 5% before field samples may be analyzed. This method was developed in UCB's laboratory (Hammond et al., 1987; Hammond and Leaderer, 1987), and we have used these passive samplers in hundreds of homes in California and thousands of homes across the U.S. The method has been successfully tested against other methods in an intercomparison study of several nicotine methods, and, in fact, was the only passive sampler to perform acceptably (Caka et al., 1990). Under routine analysis conditions, the laboratory limit of detection for the two-week samples will be 0.02 $\mu g/m^3$, although greater sensitivity is possible if needed, and we have achieved that routinely in selected studies. Field blanks will be collected routinely to ensure the samples are not being contaminated. In our experience, careful control of field sampling materials can lead to field blanks routinely having less than detectable values. The protocol for laboratory analysis of these samples for nicotine is included in Appendix B.

3.2.2 Bioaerosol Sampling

Pollen and Spores

The concentration of airborne plant pollen and fungal spores is measured most often by sampling a measured volume of air and impacting particles onto a surface that can be examined under a microscope. A trained person examines the collection surface using a light microscope and counts the numbers of different types of pollen grains and fungal spores. Collected particles are identified based on their size, shape, color, and other visible features.

The National Allergy Bureau (a division of the American Academy of Asthma, Allergy, and Immunology, AAAAI, Milwaukee, WI) has adopted the Burkard spore sampler (Burkard Manufacturing Co., Ltd., Rickmansworth, U.K.) for the stations in their sampling network. There are approximately 85 stations nationwide, 10 in California. The Burkard sampler is a wind-oriented slit impactor that can be set to operate continuously for seven days so that concentrations of airborne pollen and spores can be measured as 2-, 12-, or 24-hour averages. One such sampler will be placed at the central site (the Fresno First Street air monitoring station) to collect pollen and spore data alongside the other air sampling equipment. The Continuous Recording Burkard sampler is a similar device that can be operated indoors to collect samples onto a slide. The Continuous Recording sampler will be used in a subset of homes during the exposure intensive study to measure indoor concentrations of pollen and spores; one will also be placed outdoors at each home.

They will be set to sample for one 24-hour period, starting at midnight, during each of the three multiday sampling periods of the exposure intensive study. Thus, there will be three indoor and three outdoor samples from each of the 150 home measurements in the exposure intensive study.

The magnitude of effects and response thresholds for different aeroallergens are largely unknown, although they are expected to vary based on individual sensitivity and pollen or fungal species (Dhillon, 1991). The following AAAAI definitions of allergen concentrations allow comparisons among sampling sites across the U.S. and parts of Canada and Mexico (**Table 3-3**). The categories are based on ecological measurements rather than health effects. Other categorization of pollen and spores based on known health responses likely will be more suitable for the purposes of this study. For example, in a study of cardiac function and bioaerosol exposure in Atlanta, GA, groupings such as "pollen of the Birch family" and "small, respirable, colorless basidiospores" are being considered (personal communication, Christine Rogers, Harvard School of Public Health, Boston, MA). We will determine how well these categories apply to the data collected at the Fresno Supersite sampling location. We will develop our own categories if we determine (based on the first year's data) that the AAAAI categories do not coincide with air concentrations measured in Fresno (i.e., not detected = absent, <50% concentration = low, 50–75% = moderate, 75–99% = high, ≥99% = very high). The categories differ for specific types of aeroallergens (**Table 3-4**).

Table 3-3. Concentration categories for pollen and fungal spores and expected reactions in sensitive persons (adapted from AAAAI).

Category (Pollen/Spore Count)	Allergic Persons Who May Experience Allergy or Asthma Symptoms
Absent	None
Low	Extremely sensitive persons only
Moderate	Many sensitive persons
High	Most sensitive persons
Very high	Almost all sensitive persons, extremely sensitive persons may experience severe symptoms

Table 3-4. Concentration categories for pollen groups and fungal spores (adapted from AAAAI).

Allergen Group	Count/m^3	Concentration Category[A]
Weeds	>0–10	Low
	11–50	Moderate
	51–500	High
	>501	very high
Grasses	>0–5	Low
	6–20	Moderate
	21–200	High
	>201	Very high
Trees	>0–15	Low
	16–90	Moderate
	91–1500	High
	>1501	Very high
Fungal spores	>0–900	Low
	901–2500	Moderate
	2501–25,000	High
	>25,001	very high
[A]Categories based on the following calculations as reported by all certified counting stations in the NAB.	Median or 50th percentile (50% of counts below this concentration) 75th percentile (75% of counts below this concentration) 99th percentile (99% of counts below this concentration)	

Other methods to summarize pollen and spore data may be more relevant for the purposes of our study. For example, Delfino et al. (1996, 1997, 1998) scaled concentrations to reflect the effect at the 90th percentile of a distribution (i.e., the 90th percentile effect: the difference between the upper decile and the minimum concentration). This allowed a standardized view of effects for the fungal concentration ranges measured in their studies. Because the concentrations for any particular group can be much higher in other geographic areas or during different time periods in one region, they also presented results for fungi as the amount of change in the response variable per increase of 1000 fungal spores/m^3 of air.

Fungal Spores

More than 80 genera of fungi have been associated with symptoms of respiratory tract allergy (Horner et al., 1995) some of which are shown in **Table 3-5.**

Table 3-5. Taxonomic distribution of selected genera of allergenic fungi (Horner et al., 1995).

True Fungi	
Zygomycetes	*Mucor, Rhizopus*
Ascomycetes	*Alternaria, Cladosporium, Epicoccum, Drechslera, Stemphylium, Wallemia; Aspergillus, Penicillium; Botrytis; Fusarium; Trichophyton; Saccharomycetes, Candida*
Basidiomycetes	*Coprinus, Lentinus, Pleuotus, Psilocybe; Ganoderma, Merulius; Calvatia, Geaster; Darcrymes;* Rusts; Smuts, red yeasts (*Sporobolomyces*)
Protistan Fungi	
Oomycetes	*Phytophthora, Plasmopara* (plant downy, or false, mildews)

Measurement/Sampling

Purified allergens for sensitivity testing (e.g., skin prick tests) are available for only a few fungal species (e.g., *Alternaria alternata, Alt a* 1; *Aspergillus fumigatus, Asp f* 1; *Cladosporium herbarum, Cla h* 1, 2). *A. alternata* is one of the fungal species to which reaction is most common (Horst et al., 1990). In contrast to most aeroallergens, exposure to *Alternaria* spp. may produce particularly severe acute asthmatic symptoms, i.e., sensitivity to *Alternaria* spp. has been implicated as a risk factor for sudden respiratory arrest in adolescents and young adults with asthma (O'Hollaren et al., 1991). In the midwestern U.S., the alternaria season runs from late May into early November. Persons sensitive to fungal allergens rarely respond to a single fungal species. A study of 6000 allergy patients found that fewer than 1% are allergic to *A. alternata* only. Therefore, patient testing using available panels of fungal allergens likely underestimates the number of persons potentially responsive to spore exposures because groups of fungi known to be important allergens are not included, for example, basidiomycetes (notably *Coprinus* spp.), *Periconia, Botrytis,* hyphal fragments, and rusts) (Table 3-5) (Strachan et al., 1990; Horner et al., 1995; Delfino et al., 1996, 1997, 1998). Fungal allergens in environmental samples are detected indirectly, i.e., the presence of fungal spores or culturable fungal fragments in air or dust samples are used to indicate the probable presence of associated allergens.

The many genera and species of fungi in indoor and outdoor air can be categorized in various ways to facilitate data interpretation. Using factor analysis, Suh et al. (1992) identified correlations among housing characteristics and ecological groupings of fungi: (1) above-ground decay fungi (species of *Cladosporium, Alternaria, Epicoccum,* and *Aurebasidium*), (2) common soil fungi (species of *Aspergillus* and *Penicillium*), and (3) water-requiring fungi (species of *Fusarium*). Morey (1999) distinguished between (1) fungi from plant surfaces (phylloplane fungi: species of *Alternaria, Cladosporium,* and *Epicoccum*) and (2) soil fungi (species of *Aspergillus, Eurotium,* and *Penicillium*).

Temperature preference and moisture requirement are other useful parameters on which to stratify fungi to predict where they may be found in indoor and outdoor environments. The fungi fall into three categories with respect to temperature tolerance (Burge and Otten, 1999). Most fungi are mesophilic, with an optimum temperature range between 15° and 40°C. Psychrophiles (e.g., *Acremonium psychrophilum*) can grow at temperatures below 0°C. Psychrotolerant fungi (e.g., *Cladosporium herbarum*) have temperature minima below 15°C but also grow well above 20°C. Thermophiles (e.g., *Thermomyces* spp.) usually cannot grow below approximately 20°C. **Table 3-6** groups common fungi according to water requirement.

Table 3-6. Moisture requirements for some common fungi.

Water Requirements	Common Indoor Fungi	Comments
Hydrophilic fungi (minimum a_w>0.90)	*Fusarium, Rhizopus, Stachybotrys* spp.	Colonize continuously wet materials (e.g., soaked wallboard, water reservoirs for humidifiers, drip pans).
Mesophilic fungi (minimum a_w ≥0.8 ≤0.9, optimum a_w >0.9)	Most mycelial fungi, including *Alternaria, Epicoccum, Ulocladium, Cladosporium* spp., *Aspergillus versicolor*	Colonize continuously damp materials (e.g., damp wallboard, damp fabrics). Xerotolerant fungi can also grow under these conditions.
Xerotolerant fungi (minimum a_w <0.8, optimum a_w >0.8)	*Eurotium (Aspergillus glaucus* group), some *Penicillium* spp.	Colonize relatively dry materials (e.g., house dust at high relative humidity).
Xerophilic fungi (minimum a_w <0.8)	*Aspergillus restrictus*	Colonize very dry materials (e.g., high-sugar foods, some building materials).

Delfino et al. (1997) separated asthmatic subjects into four categories based on skin test reaction to pollen, fungi, and dust mites. There were five skin prick tests (SPTs) for fungal spores: (1) *Alternaria alternata,* (2) *Cladosporium cladosporiodes,* (3) *Helminthosporium interseminatum,* (4) *Aspergillus* spp. mix (*Aspergillus fumigatus, Aspergillus terreus, Aspergillus niger,* and *Aspergillus nidulans*), and (5) *Penicillium* spp. mix (*Penicillium digitatum, Penicillium expansum, Penicillium* [illegible]*um, Penicillium roseum,* and *Penicillium notatum*). The researchers formed analytic variables for total fungal spore and pollen concentrations and for

four additional fungal variables as follows: (1) *test fungi* — sum of concentrations of each of the five fungal genera in the SPTs, (2) *nontest fungi* — total spore concentration minus the concentration of *test fungi*, (3) *positive SPT fungi* — sum of spore concentrations for the genera in the fungal SPTs to which a subject tested positive, and (4) *negative SPT plus nontest fungi* — total fungal spore concentration minus the concentration of *positive SPT fungi*.

Verhoeff and Burge (1997) reviewed nine population-based studies that examined the relationship between allergy and the presence of fungi in the home environment. They recommended that future research intended to generate health-based data that can be used to develop guidelines for fungi in home environments should focus on susceptible populations and use measures that accurately represent exposure and adverse health effects.

Endotoxin

Airborne endotoxin can be collected on a filter, and endotoxin potency is measured by determining the biological activity of a sample in a specified assay system, such as the kinetic, chromogenic, *Limulus* amebocyte lysate (LAL) assay. This *in-vitro* biological assay is widely used to measure endotoxin because it is easy, fast, and sensitive (Levin, 1987). A dichotomous sampler has been used to collect airborne endotoxin outdoors (Monn and Becker, 1999) and could also be used indoors. Dr. Donald Milton (Harvard School of Public Health, Boston, MA) is willing to train a person in the kinetic chromogenic method he has developed to assay endotoxin in air and dust samples (Milton et al., 1992, 1997). He will provide a copy of and license for computer software needed to run the Kinetic Limulus Assay with Resistant-parallel-line Estimation (KLARE) assay. Dr. Milton has already provided sampling and analytical protocols (Appendix B). Dr. Suzanne Becker (United States Environmental Protection Agency, Research Triangle Park, NC) has agreed to test a limited number of samples using the method developed to measure cytotoxicity and induction of proinflammatory cytokines from human monocytes from endotoxin and heavy metals in fine and coarse particles in outdoor and indoor air (Monn and Becker, 1999).

Aeroallergens

Sensitization to some indoor allergens (house dust mites) has been shown to be related to the concentration of allergen in house dust of an affected person's residence. Exposure to the major indoor allergens (i.e., dust mite, cat, dog, cockroach, and *Alternaria* spp.) is estimated from the concentrations of these agents in settled dust (Platts-Mills and Carter, 1997; Platts-Mills et al., 1997). Recommended threshold concentrations for indoor allergens are based on dust rather than air samples because the amount of antigen in dust is much higher than that typically found in indoor air (e.g., µg/g versus ng/m^3) and dust samples can be collected more easily and quickly (Institute of Medicine, 1993; AIHA, 1996; ACGIH, 1999b; Rose, 1999). Aeroallergen concentration in dust is measured by immunoassay (Platts-Mills et al., 1992, 1997; Institute of Medicine, 1993; Trudeau and Fernández-Caldas, 1994). Dust samples typically are collected in a subject's bedroom and other areas where the person spends significant amounts of time indoors. Protocols developed for another study in which several of the investigators (Tager and Macher) are participating will be used for home evaluations for aeroallergen sources and house dust collection. Standard Operating Procedures (SOPs) developed for another study are available for analysis of aeroallergens in house dust (Appendix B).

GIS Coding

Geographic Information Systems (GIS) have been used to study if childhood residence near busy roads is associated with asthma (English et al., 1999). Locations of children's residences were linked to traffic count data at streets within ~1700 m (550 ft). No study appears to have assessed exposure to the combination of potential biological agents that we propose to evaluate (**Table 3-7**), which have been shown in separate studies to affect the health status of asthmatic persons. Further, research on exposures to biological agents and symptoms of hypersensitivity diseases seldom consider concurrent indoor and outdoor exposures and exposure to other airborne particles, gases, and vapors as will be done in the proposed study for a large group of graded asthmatics for multiple years.

Table 3-7. Summary of biological agents measurements.

Biological Agent	Sources	Trends/Interpretation
POLLEN - Tree (e.g., walnut, olive, ash, oak, sycamore, cottonwood, alder, birch, elm, maple, acacia, pine, eucalyptus, mulberry, willow, pepper tree, privet, juniper, almond, pecan, orange, sweet gum) - Grass (all types) - Weed (e.g., lambs quarters, sage, sorrel, plantain, ragweeds, scales, pigweed/tumbleweed, alfalfa, cocklebur)	Primarily outdoor plants. Long-scale transport from beyond neighborhoods; near-scale exposure from local vegetation.	Tree pollen typically peaks following the rainy season, approximately late January into May with little the rest of the year. Grass pollen typically peaks from approximately late March through June. Weed pollen is found throughout the year beginning in March, with various groups peaking at different times.
FUNGAL SPORES Phylloplane fungi (from plant surfaces) may include species of *Cladosporium, Alternaria,* and *Epicoccum.* Above-ground decay fungi may include these genera and species of *Aurebasidum.* Soil fungi may include species of *Penicillium, Aspergillus,* and *Eurotium.* Water-requiring fungi may include species of *Fusarium.*	OUTDOORS: fungi growing on plant surfaces and in soil (including decaying plant and animal material above ground and in soil). INDOORS: fungi growing on organic materials with water activity above 0.65	Outdoor air concentrations will reflect spore release from plant surfaces and disturbed, decomposing plant or animal materials and soil. Concentrations of phylloplane and above-ground decay fungi will be higher in indoor environments with higher outdoor air change rates. Concentrations of soil fungi will be higher in indoor environments with higher outdoor air change rates or indoor fungal growth. Concentrations of water-requiring fungi will be higher in indoor environments with indoor fungal growth.

Table 3-7. Summary of biological agents measurements.

Biological Agent	Sources	Trends/Interpretation
ENDOTOXIN lipopolysaccharides (LPS) of Gram-negative bacteria (e.g., species of *Aeromonas, Citrobacter, Enterobacter, Escherichia coli, Helicobacter, Klebsiella, Serratia,* and *Pseudomonas*)	Gram-negative bacteria in water, on plant surfaces, in soil, and associated with animals. Endotoxin concentrations exceeding, respectively, 30 and 10 times background (outdoor) concentrations will be found in indoor environments of children at increased risk, respectively, of developing and manifesting adverse health effects related to endotoxin exposure.	Outdoor air concentrations will reflect bacterial release from plant surfaces and disturbed soil and water. Endotoxin concentrations will be higher in indoor environments with higher outdoor air change rates or indoor bacterial growth.
HOUSE DUST MITE ALLERGENS (primarily *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*)	Current or past presence of house dust mites in bedding, upholstered furniture, or carpets. *Der p* 1 or *Der f* 1 concentrations >10 μg/g dust will be found in indoor environments of children at increased risk of sensitization to dust mites. *Der p* 1 or *Der f* 1 concentrations >2 μg/g dust will be found in indoor environments of mite-sensitive children at increased risk of symptoms.	Dust mite numbers fluctuate with housekeeping practices, use of acaricides, and moisture availability. Mite densities exhibit seasonal cycles that parallel humidity changes, with the highest mite concentrations occurring during periods of elevated humidity. Mites die from desiccation during long dry periods (e.g., the heating season and summers). Allergens may persist in indoor environments even after mites have died.
COCKROACH ALLERGEN (primarily *Blatella germanica* and *Periplaneta americana*)	Current or past presence of cockroaches in kitchens, bathrooms, and other indoor areas that provide adequate moisture and warmth. Detectable allergen concentrations (>5 μg/g) will be found in indoor environments of children at increased risk of sensitization to cockroaches and of cockroach-sensitive children at increased risk of symptoms.	Cockroach numbers fluctuate with housekeeping practices, use of pesticides, and moisture availability.
Mammalian allergens (primarily *Felis domesticus* and *Canis familiaris*)	Pet cats and dogs, environments inhabited by pets, and allergenic particles carried by humans in contact with animals or allergen-contaminated environments. *Fel d* 1 or *Can f* 1 concentrations >8–10 μg/g dust will be found in indoor environments of children at increased risk of sensitization to cats or dogs.	Cat and dog allergens will be higher in indoor environments in which cats and dogs currently are found and those visited by persons in contact with animals. Allergens may persist in indoor environments even after animals are no longer present.

3.3 TASK 1: PROTOCOL REFINEMENT

The exposure assessment part of this study requires interaction and coordination with myriad other groups: the project sponsors, the advisory board, the investigators from the health study, the field team for the health study, the field team for the central site measurements, the field team for CRPAQS measurements, and the ARB mobile van operators. Within the exposure assessment team there must be coordination not only among field operations, laboratory analyses, and data management, but also between the measurements and the modeling aspects of the study.

The exposure assessment team will work closely with the health team throughout the study to ensure that the data generated will intermesh and be most useful for the overall study of the relationship between air pollution and asthma in children. The exposure assessment team and the health team will work with the project sponsors and the project advisors to refine the overall project objectives and determine the specific exposure-assessment information required to meet those objectives. Meetings with the California Air Resources Board, the advisory board, and the study investigators will take place annually at the University of California, Berkeley. The investigators in the health study (Part A) and the exposure assessment study (part B) will meet at least quarterly at the University of California, Berkeley, to coordinate the study; although there will be more frequent meetings in the first year of the study.

At the beginning of the project, the focus will be on finalizing the protocols for the central site measurement augmentations and the routine microenvironmental monitoring in the home. Within a few months, the focus will shift to refining the neighborhood site measurements to be made during CRPAQS in December and January of the first year. A few months after that, the priority will be to refine the protocols for the exposure intensive microenvironmental sampling in the homes and the personal sampling. Then, the protocols for using the mobile van for neighborhood and school measurements will be refined. Throughout, the modeling protocols will be discussed to ensure that the measurement strategy continues to provide the data necessary for the modeling. The final refinements of the modeling methodology will be completed in the third year.

The health study plans to begin the two-week panels in August of 2000, so both the routine microenvironmental measurements to be made at the childrens' homes and the biological measurements to be made at the central site must be ready to start at that time. The health study will begin before the exposure assessment component, so this will require careful planning and coordination. For the routine home microenvironmental sampling, the exposure assessment team will be responsible for writing the protocols for sample handling, placement of samplers in the homes, recording field data, retrieving samples, proper storage, and packaging and shipping. The exposure assessment team will train the health team in these areas, as the health team will have the responsibility for conducting the field operations for this part. During this time the questionnaires and diaries associated with the routine measurements during the two-week panels will be finalized, formatted, and printed.

The biological measurements which are planned for the central site and the neighborhood sites during CRPAQS will include pollens, fungal spores, and endotoxins. The samplers for these must be set up, field staff trained, protocols written, and sampling forms developed. All of

this must be coordinated with the other measurement programs at the central site and the CRPAQS sites. Very early in the study we will meet with the staff at the central site and the CRPAQS neighborhood sites to coordinate the measurements we plan to add to the central site and the neighborhood sites. We plan to set up the Burkhard samplers and the endotoxin samplers at these sites, and periodically maintain the ones at the central site in the first two years, but we will rely on the staff at these sites to change the filters and slides, store the collected samples, and deliver them to the health team to mail them to our laboratory for analysis. The logistics of this will be worked out jointly with the health team, the field staff at the Fresno central site, the field staff at the CRPAQS sites, and the exposure assessment team. As the slides are read for pollens and spores, and the endotoxin analyses for the first year are completed, patterns will begin to emerge, and the protocols may be revised and tailored for the Fresno area and this health study.

Protocols will be written for sampling and analyzing each agent for the study. Quality assurance plans must be detailed and implemented. Details of and protocols for data management will be developed during the refinement phase. Supplies and equipment must be purchased. The equipment will tested, modified in some cases, and calibrated.

As measurement data are evaluated, the modeling will be affected, and preliminary modeling results may lead us to change the measurement priorities. Furthermore, we have built into the project preliminary health study results that may guide refinements in the measurement and modeling components. These will all be discussed with the exposure assessment investigators, the health effects investigators, the project sponsor, and the advisory board.

3.4 TASK 2: AUGMENTATION OF CENTRAL AND NEIGHBORHOOD SITE MEASUREMENTS DURING CRPAQS

Our technical approach to obtaining the ambient data we need to meet our exposure objectives will involve supplementing existing measurements. There will be a large ambient air quality data set collected at a central site and several neighborhood sites in the Fresno area over the next five years. We will take advantage of these measurements and add to them, as needed, to meet our objectives.

The routine gaseous and particulate-matter monitoring and sample collection being performed at the ARB Fresno-First Street site as part of CRPAQS and of the EPA-sponsored PM Supersite network will provide an invaluable data set for many agents of interest in our exposure study. In addition, these measurements are expected to continue for five years (or more). These measurements include hourly (or sub-hourly) continuous monitoring for many gaseous and particulate-matter agents, along with integrated sample collection and laboratory analysis for additional agents. At the central site, we will augment the measurements with daily-average spore, pollen, and PM_{10} endotoxin measurements. Thus, the CRPAQS/EPA Supersite data, plus our supplemental data, will provide the central site data we need as the basis for estimating each child's daily exposure during the two-week health panels.

In addition, CRPAQS will conduct a two-month winter intensive-sampling program in December 2000 and January 2001. This two-month program will provide data for many of the

same agents as the Fresno Supersite data at four additional neighborhood sites in the Fresno area (see the second column in Table 3-1 for those measurements of interest for this study). The filter samples will be collected on 15 days selected for the potential for high PM concentrations. On these 15 days, we will augment the measurements with daily-average spore and pollen measurements, and with PM_{10} endotoxins. The detailed neighborhood data will support our understanding of neighborhood variability for each of the agents of interest.

Thus, with a modest investment for supplemental spore, pollen, and endotoxin measurements, we will obtain hourly or daily data at a central site for a wide range of agents of interest. We will obtain similar data at four neighborhood sites for 15 days during two months in the winter. See Table 3-1 for a list of the agents and the time resolution of the data.

3.5 TASK 3: ROUTINE MICROENVIRONMENTAL MEASUREMENTS

3.5.1 Passive Measurements in Homes

As mentioned in Section 3.1, if we can't directly measure personal exposure of all children in the study, we would like to measure all agents of interest in every microenvironment possible. This is very expensive. However, simple-to-install passive samplers can be used to meet some of the exposure objectives for several agents. Passive samplers can identify and characterize indoor sources of NO_2 and ETS. Passive samplers also can characterize average indoor/outdoor ratios for ozone in homes. In addition, the health team (Part A) will be visiting each of the 50 homes during each two-week panel throughout the five-year study. Thus, our technical approach involves the routine measurement of NO_2, ETS, and ozone at homes using passive samplers installed by the health team. The ozone samples will only be collected during the high-ozone season (May through September), since ozone concentrations indoors will be very small during the non-summer period.

In addition, as part of the two-week panels, the health team will collect one integrated house-dust sample in three or four rooms of each house, using a special vacuum cleaner. The combined samples from each house will be analyzed for house-dust allergens (dust mites, dog, cat, and cockroach allergens) and endotoxins. We will do this three times for each home in the two-week panels during years 1 and 2. Additional samples will be collected and analyzed during years 3-5 if a family moved, acquired or lost a dog or cat, or a child developed increased or new sensitization to asthma.

3.5.2 Home Characteristics Surveys

Housing characteristics for all of the study participants are needed for a variety of purposes, including (1) for direct evaluation of associations between housing characteristics and children's respiratory health that have been found in other studies; (2) for selection of homes to include in the intensive sampling program, and (3) for use in the data analysis and exposure modeling. Housing characteristics will be obtained from questionnaires completed by the parents of all participants and from direct observation during home visits by the technicians that install and retrieve the passive sampling devices. Questions will include type of housing,

housing age, use of gas appliances, wood-stoves, heating systems, air conditioners or cleaners, window openings, humidifiers, mattress covers, pets, pests, dampness and fungi, carpeting, household crowding, cooking habits, cleaning habits, presence of smokers in the home, and other factors that may influence allergen levels, indoor air quality, and indoor/outdoor pollutant relationships. A brief home assessment walk-through will be undertaken during the first panel period each year for each child and will use protocols similar to those used in other studies. A sample home inspection form developed by our team is included in Appendix B (CHAMACOS Home Inspection Form). As indicated by this example, our recommended approach is to use comprehensive survey instruments that may be prone to gathering too much information rather than too little information. The specific questionnaires and protocols to be used in the study will be established at the beginning of the study, and will be based on the project team's experience and a review of survey instruments and inspection protocols used in other studies.

3.6 TASK 4: EXPOSURE INTENSIVE MEASUREMENTS

Detailed data for the agents of interest are needed indoors and outdoors at as many homes as possible and for as many time periods as possible. However, it is expensive and time consuming to perform sampling in all 450 homes in this study one time, much less multiple times. Some of the microenvironmental measurement needs and requirements are listed below:

- Indoor samplers must be quiet and as non-invasive as possible.
- Multiple sets of data for a given home are needed for the agents that vary at the home level.
- Data are needed most during the periods of the day when the child is likely to be at home.
- The methods and time resolution of the home data must match those for the central and neighborhood sites.
- Separate data for weekdays and weekends are desired.

In order to meet as many of these needs and requirements as possible, we have designed a sampling strategy to collect integrated or continuous data both inside and outside of the homes for the agents of interest using either passive badges, active badges, integrated filter samplers, or continuous monitors. We will use two field technicians to sample at six homes during each of 25 two-week panels during year two, for a total of 150 home visits. This will allow us to sample twice at 54 homes in two different seasons and once at 46 other homes.

Selection of the subjects and homes will be based on location of residence, housing characteristics, and household smoking status to define spatial variation in air pollution levels in the study area while taking into account exposure-modifying factors, such as surrounding vegetation and proximity to roadways with heavy vehicular traffic. This design will assure that the modeling will be based on data that are representative of the entire study population (e.g., in terms of location and types of residence, types of activity patterns, relative location to central monitors and to fixed sources of ambient air pollution) and the range of air pollutant conditions that can be expected to occur throughout the study period.

For PM mass and chemical composition, we will obtain integrated data for each of three periods during the two weeks: Tuesday 4 p.m. to Friday 8 a.m. (excluding 8 a.m. to 4 p.m. daily), Friday 4 p.m. to Monday 8 a.m., and Monday 4 p.m. to Thursday 8 a.m. (excluding 8 a.m. to 4 p.m. daily). Note that the weekday samples only include the hours when the children are expected to be at home. In contrast, the weekend samples include all hours, since the children are often at or near home on the weekend.

For NO_2 and ozone, we will obtain 12- to 14-day indoor and outdoor samples using passive badges. For ETS, we will collect indoor samples for the three periods at all homes with smokers and some of the smoke-free homes using an active filter sampler, but only analyze one-third of the samples collected. For indoor and outdoor $PM_{2.5}$ mass, $PM_{2.5}$ ion, $PM_{2.5}$OC, $PM_{2.5}$EC, PM_{10} mass measurements, and PM_{10} metals we will use a timed, multi-leg filter sampler similar to the two-week sampler designed and used for the Southern California Epidemiologic Study sponsored by ARB. For indoor and outdoor particle scattering measurements, we will borrow nephelometers from CRPAQS and collect hourly particle scattering data for each of these 150 home visits.

3.7 TASK 5 – PERSONAL, NEIGHBORHOOD, AND SCHOOL SAMPLING PROGRAM

The personal sampling component of this study is limited to a simple validation of the models developed to estimate personal exposure from microenvironmental samples and time-activity diaries. Although personal sampling is theoretically a better approach, it is impractical for this study for many reasons: 1) personal sampling systems to measure all the contaminants of interest do not exist; 2) even if such systems could be constructed, measuring each child's exposure to the agents of interest on each day for ten two-week panels would generate over a million samples, and cost hundreds of millions of dollars to collect and analyze; and, 3) children cannot be asked to wear the sampling systems for even a reduced set of air contaminants for two full weeks during each panel, i.e., two to three times per year. Therefore, our goal remains to estimate each child's daily exposure to the agents of interest through the use of a model incorporating 1) the daily variability measured at the central site; 2) the spatial variability evaluated with measurements at five sites in Fresno during the CRPAQS winter intensive study, with the exposure-intensive measurements made during 150 use visits, and with measurements made by the ARB mobile vans; 3) home-specific characteristics determined by measurements, observations, and diary; and 4) diary information about time spent in various locations and activities (e.g., smoking, use of wood stoves or fireplaces) occurring in those locations. A small set of personal samples will be collected solely for the purpose of evaluating the model estimates for seven agents: NO_2, ozone, PM_{10} mass, $PM_{2.5}$ mass, PM_{10} endotoxin, $PM_{2.5}$ endotoxin, and ETS.

Twenty-five children, age 8 or older, will be selected for personal sampling from homes where the exposure-intensive sampling is being conducted; the personal sampling will be conducted during the two-week exposure-intensive sampling. The children and the seasons sampled will be selected to maximize the dynamic range of concentration in order to evaluate the model with the minimum number of samples. Each child will wear the backpack sampler for 48 hrs.

The personal sampling system will consist of passive monitors for NO_2 and ozone and a newly developed, battery-operated personal particle sampler with size selection that separately collects particles less than 2.5 μm and those between 2.5 and 10 μm (note that the cut point can also be 1 μg if we so choose). Mass and endotoxin will be measured for each of the two particle sizes collected; and nicotine, a tracer for ETS, will be measured on a filter placed downstream from the filter collecting fine particles.

This sampler has a newly developed inlet that solves some of the sampling artifacts from previous studies; that is, it does not collect fibers or dandruff, which are excluded before the impactor. The pump is very quiet and has been found to be acceptable to children 8 years of age and older who have worn it in a backpack arrangement without complaint.

An ARB-equipped and -operated mobile-monitoring van will perform 2- to 7-day monitoring and sample collection during Years 2 and 3 at selected neighborhood and school sites. The ARB has plans to equip several monitoring vans for deployment in various locations of interest in California. The monitoring vans are expected to contain continuous monitors for gaseous and PM agents such as ozone, NO and NO_2, particle scattering, $PM_{2.5}$ and PM_{10} mass, $PM_{2.5}$ OC and $PM_{2.5}$ EC, and $PM_{2.5}$ sulfate and nitrate. The vans are also expected to contain filter samplers for daily $PM_{2.5}$ and PM_{10} mass, ions, and metals concentrations; an additional filter sampling leg could be used to collect samples for PM_{10} endotoxins. After the exposure field intensive study is over, we could also provide samplers for spores and pollens to be installed in the vans. Based on preliminary data analyses, we would select several neighborhoods with a potential of significant health agent impacts and recommend that a sampling van be deployed in those neighborhoods for a period of 2 to 7 days each. We would also identify and recommend several schools in the area for mobile sampling. We assume that ARB would operate the sampling van, perform laboratory analyses, and process and quality-control the sampling-van data.

3.8 TASK 6 - DATA ANALYSIS AND EXPOSURE MODELING

The technical approach for data analysis and exposure modeling is designed to improve our understanding of air quality and children's personal exposure in Fresno. The proposed analyses are needed to test our hypotheses and to develop a method (model) to estimate the daily air pollution exposures of the study participants over the five-year period. The method will be developed and evaluated using data primarily collected in the first two and one half years of the study. The technical approach involves conducting exploratory data analyses that will lead to specific sub-models of relationships. The sub-models will be combined into an overall model (and database) of the children daily exposures for all of the agents listed above.

Conceptually, we expect the exposure model will use (1) daily central-site data, (2) relationships between central-site concentrations and outdoor concentrations at specific homes, in specific neighborhoods, and/or in different types of neighborhoods, (3) indoor/outdoor relationships for specific homes and/or types of homes, (4) house-specific data for indoor concentrations of selected agents, and (5) time-activity data for individual participants. Model testing will be conducted using the personal exposure data to assess model performance. The

proposed analyses are organized according to these six elements. This analysis plan incorporates all of the "analysis"-specific aims listed in Section 1.3.

3.8.1 Analysis of Central-Site Data

The central-site air quality and meteorological measurements will be examined in order to understand conditions at the site, to reconcile the different measurement techniques, to understand the temporal variability of gases and particles, and to understand relationships between gases, particle mass, particle chemical composition, particle size, and particle number densities in Fresno. For model building purposes, it is important to identify the pollutants that are collinear and those that have distinct temporal variations. These data will provide a unique opportunity to examine the relationships between infrequently measured chemical and physical particle characteristics and the routinely collected parameters. This applies not only to how particle size and number vary with PM mass and conventional components (SO_4, NO_3, EC, and OC), but also to how concentrations of pollens, fungal spores, and endotoxins vary with PM_{10}. Summary statistics, correlation analysis, time-series plots, scatter-plots, and box-whisker plots will be used to understand these data. Seasonal, monthly, daily, day-of-week, and diurnal displays of the concentrations will be examined to characterize the temporal variability. These analyses will satisfy Specific Aim No. 2: "To evaluate the daily variability of Group I (regional) and Group II (neighborhood) agents using the Fresno supersite air quality data."

3.8.2 Analysis of Neighborhood Data

The data collected at a variety of neighborhood locations in this study will provide a unique opportunity to characterize the within-community variability in outdoor pollutant concentrations. We plan to analyze the spatial and temporal variability in these data. Our expectation is that the concentrations of Group I species will show little variation within the community, and Group II species will show some bias from the central site. The direction and extent of the bias is important to capture and include in the exposure model as one of the principal means of improving the accuracy of the model.

The variety and amounts of neighborhood-scale data sets will enhance this analysis. For example, during the two-month CRPAQS winter intensive study, concentration of PM mass, PM chemical components, selected gases, pollens, spores, and endotoxins will be available from the central site and four other community-monitoring stations in the Fresno/Clovis area. During the one-year intensive sampling program, continuous (hourly) light scattering and integrated ozone, NO_2, and PM concentration data will be available outside 96 different homes. Two-week integrated ozone concentrations will be available outside 450 homes for two different periods from May through September from the routine panel studies. In addition, the ARB van(s) will provide continuous, as well as integrated, measurements of outdoor concentrations and particle number density at selected schools and homes in the community. We plan to examine all these data sets and look for consistency in results from the different types of data.

In the first year of the study, we plan to categorize the neighborhoods of all 450 participants. They will be categorized with respect to probable mobile source activity and vegetation. Land-use, vegetation biomass, and average daily traffic volumes on moderate and

heavily traveled roadways (from CALTRANS) will be displayed along with residence and school locations in a Graphic Information System (GIS). This information will be used initially to select homes for the intensive home monitoring study and subsequently used as part of the analysis of the neighborhood-scale pollutant data.

Two initial steps in dealing with the pollutant database are to calculate the concentrations from the continuous data for the averaging times of the integrated samples and to map the data. A database will be set up for this analysis with as many pollutants and locations as possible with concentrations for comparable averaging times. We have found that there is no good substitute for looking at the patterns in the data early in the exploratory analysis; thus, spatial plots of the integrated data and comparable central-site data will be developed. Correlations and regressions will be performed to assess how well the neighborhood-scale concentrations track the central-site concentrations. The magnitude of the bias between specific neighborhood location concentrations and the central-site concentrations will be examined. The high time resolution data, such as the light scattering from nephelometers outside houses, will be compared to the central-site data to assess how the bias varies with time of day and/or day of week. The diurnal dependence will be examined to determine whether the deviations from the central-site data are consistent and whether they follow expected trends (e.g., the morning and evening increases from mobile source activity). We will assess how well various factors, such as proximity to roadways and traffic density and type and extent of vegetation, explain the variance in concentrations across the city.

The coherence of results for different species will be examined. We expect to find consistency in species that are co-emitted from major source types. Analysis of the spatial patterns and seasonality of the bioaerosols and the other pollutants will be particularly informative because concurrent data for these species have rarely been measured in a manner that would allow objective comparisons. We expect to find significantly different spatial patterns (or neighborhood-scale variations) for bioaerosols than $PM_{2.5}$ or PM_{10} mass. Even among the bioaerosols, significant differences are likely between pollens, spores, and endotoxins. We are hoping to find reasonably coherent spatial patterns. However, regardless of the extent of coherence, we will have valid comparisons between the neighborhoods of the study participants and the central site on which to base a model. The temporal and spatial correlation analysis for the different species will give us information on the explanatory power of different types of models under consideration.

3.8.3 Analysis of Indoor/Outdoor Pollutant Relationships

The data from the indoor and outdoor sampling at participants' homes will be analyzed and categorized to understand how much variation exists in the homes of our study population. The housing questionnaire data will be used in the analysis to determine which characteristics explain differences in indoor/outdoor (I/O) ratios and concentrations. We will explore associations between I/O ratios and ventilation characteristics, which will affect all pollutants. We will also examine relationships between indoor pollutant levels and probable sources, including NO_2 and gas-appliance use; ETS and smoking; pollens and houseplants; and $PM_{2.5}$ and smoking, cooking, cleaning, and pets. From these analyses, we expect to develop representative models of how much outside air infiltrates the indoor environment (expressed as I/O ratios for

different pollutants and types of structures) and how much indoor sources typically contribute to indoor concentrations. These relatively simple models will be constructed so that we can apply them (using housing questionnaire data) to all of the study participants.

3.8.4 Analysis of House-Specific Data for Indoor Concentrations of Selected Agents

The Group II and III (e.g., ETS) pollutants will be analyzed to determine which pollutants are primarily indoor contaminants, with little relation to outdoor air. The indoor measurements of house dust allergens and ETS data are being collected to use directly in the exposure model; thus, it will not be necessary to develop models of their concentrations. Fungal spores and endotoxins may behave either as Group II or Group III pollutants, and we need to know which participants have homes with high indoor concentrations that are likely to dominate their exposures. We are also interested in which household characteristics may explain the variability of indoor concentrations. Thus, we will conduct correlation and regression analysis (as described above) to assess the relationships. These will not only be interesting but could be useful if there is a need to fill in missing data and/or extrapolate the data to other time periods or populations.

3.8.5 Analysis of Time-Activity Data for Individual Participants

Time-activity questionnaire data will be collected on a daily basis during the panel studies. These surveys will provide a wealth of information about the individuals in the study. Our analysis will partially be guided by previous findings from large-scale studies, such as the 1992-1994 National Human Activity Pattern Survey (NHAPS) (Nelson et al., 1994; Robinson and Blaire, 1995) and the 1988-1990 California Activity Pattern (CAP) Survey (Wiley 1991). These studies have consistently shown that children, on average, spend more time outdoors on weekend days than on week days and more time outdoors in the summer months than at other times of the year (Klepeis et al., 1996; Tsang and Klepeis 1996). They spend the most time indoors at their residences and a substantial amount of time indoors at schools

We plan to analyze these data by type of day (weekday versus weekend) and season (summer versus non-summer) for the fractional time spent in the following locations:

1. Residential – indoor
2. Residential – outdoor
3. School – indoor
4. School - outdoor
5. In vehicle
6. Near vehicle(s)
7. Other outdoor
8. Office/factory
9. Mall/store
10. Church/public building
11. Restaurants
12. Other Indoor

The means and variance of time-use within subjects and between subjects will be examined. After these detailed data are examined, the locations probably will be aggregated into a smaller number that will be practical for modeling. The microenvironments for which we will have pollutant data are the first four types listed. However, it is important to know how individuals spend their time; if we find that some children spend a great deal of time in vehicles, restaurants, or malls, we may incorporate these data and submodels (or adjustment factors) from the literature to estimate microenvironmental concentrations for locations that are only important for certain children.

It is not clear from the literature whether the time-use of asthmatic children is noticeably different from non-asthmatic children. While this question is interesting and may provoke some comparisons of these data with national surveys, it will not be the focus of this analysis.

3.8.6 Model Assembly and Testing

Modeling software is needed to estimate the daily personal exposures of the study participants to the agents. STI has developed exposure modeling software for general population exposure assessment (REHEX-II, Lurmann and Korc, 1994) and for cohort exposure assessments where subject-specific information is available (Lurmann and Kumar, 1998). The software provides a general framework that can be modified to suit the needs of particular applications. The software is structured to use a data-driven approach to exposure assessment.

The recommended technical approach will be to modify STI's existing FORTRAN exposure modeling software to interface with the subject-specific data available for the Fresno cohort and to incorporate the specific submodels that show the greatest explanatory power. The model will be interfaced to the housing questionnaire data, the time-activity data, the central-site ambient air quality data, the house-specific data for Group III agents, and a library of microenvironmental concentration submodels. The framework will allow alternative submodels to be easily tested.

After the interface has been established and debugged, preliminary sensitivity analyses will be conducted to identify important parameters (or perhaps unimportant parameters). Some model refinements may be made to make better use of the available data, based on these sensitivity results. Next, the model will be tested against the $PM_{2.5}$ and $PM_{2.5-10}$ personal exposure data collected on the Fresno subjects. These data are quite limited but will provide a reality check for the model. Our expectation is that we can develop models that explain more than 50 percent of the variance in personal PM exposures. The newer personal monitoring devices that will be used to collect the personal data are much less likely to collect skin flake, clothing lint, dandruff, and other substances that we believe exaggerated personal cloud effects and probably confounded some earlier comparisons for PM_{10}. The small number of samples, subjects, and pollutants for which the model can be tested will limit the extent to which we can make statistical statements regarding the model performance. Much of the support for the model validity will be derived from the actual data it will use and from the statistical analysis that supports particular submodels that will be incorporated into the overall model. Given that the submodels will be developed from data from participants' homes and schools, we believe the exposure modeling approach will yield more accurate estimates than previous studies.

3.9 TASK 7: QUALITY ASSURANCE

In general, the quality assurance/quality control (QA/QC) activities are designed to ensure that high-quality data will be generated and delivered to the exposure data analysts and modelers and to the health team. Thus, the QA/QC activities will involve all aspects of the exposure portion of the project, including field sampling and monitoring, chain-of-custody of samples, laboratory analysis, data validation, and data management. Specific activities will include the use of standard operating procedures (SOPs) and logbooks, regular calibrations, chain-of-custody forms, interlaboratory comparisons, and systems audits of the field sampling and monitoring procedures and of the data management system.

3.10 TASK 8: DATA MANAGEMENT

In general, the data management activities are designed to provide the capabilities needed to support the data validation, data analysis, and exposure modeling tasks, as well as to deliver the validated data to the heath team. The data management activities will include the design and construction of a Microsoft ACCESS database with the required data validation, data display, and data output capabilities, ingest and review of project and CRPAQS data, validation of exposure data, and the delivery of exposure data to those performing exposure analysis and modeling, and to the health team.

3.11 TASK 9: DOCUMENTATION

The activities in this task are designed to provide planning and progress information to the sponsor and to other team members. As part of this task, we will provide regular updates on exposure team plans and activities (via written monthly progress reports and presentations at quarterly project-team meetings and at annual project-review meetings). In addition, we will prepare a draft and a final report on the exposure work performed for this project and the exposure team results. The results to be included in these reports will use the data collected during the first two to three years of the study, as described in this proposal and in the refined Protocol. We will also prepare several manuscripts on the exposure results for publication in scientific journals.

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4. STATEMENT OF WORK

4.1 TASK 1: PROTOCOL REFINEMENT

The Protocol refinement task will include the following components:

- Work with the health team, the project sponsors, and the project advisors to refine the overall project objectives and the specific exposure-assessment information required to meet those objectives.
- Develop further details of each measurement task (Tasks 2, 3, 4, 5, and the measurement portion of Task 7) and list the equipment, supplies, and personnel required to perform those measurements.
- Coordinate our planned measurements with other measurement programs in the Fresno area, especially with the Fresno Supersite and CRPAQS programs.
- Develop further details of the planned data analysis and exposure modeling. Focus on how the analysis and modeling results will provide the required information to the health team and on the data needs for both analysis and modeling.
- Develop further details of the quality assurance and data management tasks. Focus on the data required by the health team and how and when those data will be provided to the team.
- Complete additional planning of the project schedule and how and when the various tasks and expected task results will interact and influence each other.
- Prepare a draft and final Protocol to guide the remainder of the project.

In addition, we will prepare for and participate in quarterly project team meetings and annual project review meetings.

4.2 TASK 2: AUGMENTATION OF CENTRAL AND NEIGHBORHOOD SITE MEASUREMENTS DURING CRPAQS

4.2.1 Central Site (Fresno Supersite)

Routine gaseous and particulate-matter monitoring and sample collection are being performed at the ARB Fresno-First Street site as part of CRPAQS and the EPA-sponsored PM Supersite network. These measurements include hourly (or sub-hourly) continuous monitoring for many gaseous and particulate-matter agents, along with some integrated sample collection for later analysis in the laboratory (see the first column in Table 3.1 for those agents of interest for this study). We will augment these measurements with the following:

- Daily-average spore and pollen measurements. We will use a Burkard sampler to collect a continuous slit-shaped trace on a tape each week. The tape will be cut into daily sections and mounted on glass slides. The slides will be read in the laboratory to produce daily averages. We will begin collecting these samples when the first health panel is

enrolled (August or September 2000) and will continue until the last panel is completed (about September 2004). We will analyze the weekly slides for daily averages on each day that a panel is being evaluated through the end of Year 2 (June 2002), for a total of 493 daily averages, including 10 percent duplicates and blanks.

- Endotoxins. Using an eight-day sequential filter sampler, we will collect daily (24-hr average, midnight to midnight) filter samples for later processing and analysis. We will begin collecting these samples when the first health panel is enrolled (August or September 2000) and will continue until the last panel is completed (about September 2004). We will analyze the daily filters from the beginning of the project through the end of Year 2 (June 2002) on each day that a panel is being evaluated, for a total of 493 daily averages, including 10 percent duplicates and blanks.

Note that we expect support from the Fresno Supersite operator (ARB) for once-per-week filter changing and shipping.

4.2.2 Neighborhood Sites During CRPAQS Intensive-Sampling Program

CRPAQS will conduct a two-month winter intensive-sampling program in December 2000 and January 2001. These measurements will include hourly (or sub-hourly) continuous monitoring for many gaseous and particulate-matter agents, as well as daily integrated sample collection, at four additional neighborhood sites in the Fresno area (see the second column in Table 3.1 for those measurements of interest for this study). The filter samples will be collected on 15 days selected for the potential for high PM concentrations. On these 15 days, we will augment the measurements with the following at each of the four sites:

- Daily-average spore and pollen measurements. We will use an indoor Burkard sampler (protected by a small shelter for outdoor use) to collect samples to be read in the laboratory to produce daily averages. We will collect and analyze the samples for a total of 15 daily averages at the four sites, plus 10 percent duplicates and blanks, for a total of 66 samples.
- Endotoxins. Using a filter sampler, we will collect daily (24-hr average, midnight to midnight) filter samples for later processing and analysis. We will collect and analyze the daily filters for the 15 days at each of the four sites, plus 10 percent duplicates and blanks, for a total of 66 samples.

4.3 TASK 3: ROUTINE MICROENVIRONMENTAL MEASUREMENTS

The health component of the study (Part A) will begin with two-week testing periods for each of the nine panels in August or September 2000; the panels will each continue to be tested for two-week periods until early in Year 5 of the study. During the time when each panel is tested for the first time, we will provide to the health team passive samplers for NO_2, ETS, and ozone. The health team will install samplers for NO_2 and ETS inside each home and, for ozone, inside and outside each home. The samples will be exposed for about twelve to fourteen days during the two-week testing of each panel.

We will provide and analyze indoor samples of NO_2 for all homes in all panels during Years 1 and 2, (50 homes in 32 panels for 1,600 samples), plus 10 percent blanks and duplicates, for a total of 1,760 NO_2 samples. For ozone, we will provide and analyze both indoor and outdoor samples for each home (450 homes, indoors and outdoors, twice) for 1,960 total samples, including 10 percent blanks and duplicates. The ozone samples will only be collected during the high-ozone season (May through September); thus, the ozone samples will be collected twice at each home during the summers of 2000, 2001, and 2002.

For ETS, we will provide samples and perform analyses for all home visits during each two-week panel for the first two years, as well as provide samples for the remaining years. We will analyze a subset of these samples collected in the first two years (see below). The unanalyzed samples are stable and can be stored for analysis later. We will seek additional support from other sources at a later time to analyze the samples collected in Years 3 through 5, using the same prioritization outlined below for the first two years of samples. During the first two years, we will provide samples and perform analyses for two samples inside each home, plus up to 50 percent of the remaining home visits, for a total of 1,227 ETS measurements, including 10 percent blanks and duplicates. The selection of the additional 50 percent will be based on the following:

- Homes with resident smokers
- Homes in which the measured concentration of nicotine is greater than 0.1 $\mu g/m^3$ in either of the two measurements
- Households without a resident smoker but which report visiting smokers during the sampling period
- Homes that gain a resident smoker
- Any home not previously tested (when a student moves or smokers enter the household, for example)
- A fraction of the smoke-free households

The health team will place and remove the NO_2, ozone, and ETS passive samplers and will ship them to the appropriate laboratory. The ETS samples will be analyzed in the UCB laboratory. For NO_2 and ozone, we will use the laboratory recommended by the manufacturer of the passive badges: Research Triangle Institute (RTI).

In addition, as part of the two-week panels, the health team will collect one integrated house-dust sample in three or four rooms of each home, using a special vacuum cleaner. The combined samples from each home will be analyzed for house-dust allergens (dust mites, dog, cat, and cockroach allergens) and endotoxins. A subset of the samples collected will be analyzed as follows:

- All homes in all panels three times in Years 1 and 2 (1,485 samples, including 10% blanks and duplicates)
- During Years 3 and 4, up to 10 percent additional samples (180) from homes of those who moved to a new residence, who acquired or lost a cat or dog, or who developed increased or new sensitization to allergens.

Thus, we will analyze a total of 1,665 samples for house-dust allergens and endotoxins, including 10 percent blanks and duplicates. These samples will be analyzed in the UCB laboratory.

4.4 TASK 4: EXPOSURE INTENSIVE (MICROENVIRONMENTAL) MEASUREMENTS

During Year 2, we will perform additional detailed measurements at 96 of the 450 homes in the health study; we will sample twice at 54 homes in different seasons and once at 42 other homes. We will perform sampling at six homes during each two-week panel over a period of about one year (about 25 two-week periods). These measurements will include those in the following list. Indoor and outdoor PM mass and composition samples will be collected for each of three multi-day sample periods during each two-week health panel; the periods will not include the weekday (8 a.m. to 4 p.m.) periods when students are not expected to be home. The periods will be Tuesday 4 p.m. to Friday 8 a.m. (excluding 8 a.m. to 4 p.m. daily), Friday 4 p.m. to Monday 8 a.m., and Monday 4 p.m. to Thursday 8 a.m. (excluding 8 a.m. to 4 p.m. daily).

- NO_2: We will collect ten- to fourteen-day outdoor passive samples for NO_2 to supplement the routinely collected indoor samples (Task 3 above). This will result in 165 additional samples, 150 for each home visit plus 10 percent blanks and duplicates.
- ETS: We will collect active ETS samples downstream from the particle samplers for the three periods at all homes (450 collected samples). We will select one-half of those samples to analyze including each household where a smoker resides (assumed to be one-third of the homes) and 25 percent of the smoke-free homes, plus 10 percent blanks and duplicates, for a total of 248 analyses.
- Endotoxins: Using a filter sampler, we will collect (on a midnight–to-midnight schedule to match the central site) one multi-day sample indoors and one outdoors during each home visit for later processing and analysis. We will analyze these filters for a total of 330 samples, including 10 percent blanks and duplicates.
- Pollens and spores: We will use an indoor Burkard sampler to collect one 24-hr (midnight-to-midnight) sample indoors and one matching sample outdoors during each of the three sampling periods. Thus we will analyze these matched indoor and outdoor 24-hr pairs, for a total of 990 samples, including 10 percent duplicates and blanks. These samples will be analyzed in the UCB laboratory.
- PM mass and chemical composition: We will use multi-leg filter samplers (similar to the two-week sampler designed and used for the USC/ARB Children's Health Study) to collect indoor and outdoor $PM_{2.5}$ mass, $PM_{2.5}$ ion, $PM_{2.5}OC$, $PM_{2.5}EC$, and PM_{10} mass measurements. We will analyze all the mass and OC/EC sample pairs but only one sample pair for sulfate, nitrate, and ammonium ions from each home visit. The mass and ion measurements will be made at UCB while the OC/EC indoor/outdoor measurements will be made at Desert Research Institute (DRI).

- PM_{10} metals: Especially because of the need to exceed the detection limits for metals of interest as often as possible, we will not change the filter to be used for metals analyses while sampling during the three periods at each home. Then we will have the laboratory analyses performed at DRI for an average over the three periods (excluding weekdays, 8 a.m. to 4 p.m.) for a total of 165 multi-day PM_{10} metals samples.
- Particle scattering: After February 2001, we will borrow twelve Radiance Research nephelometers from CRPAQS and deploy them during each of the 150 home visits. We will collect hourly particle scattering data both indoors and outdoors for each of these 150 home visits.

Note that we expect to miss or lose about 10 percent of the home samples for each agent, due to the probable inability to access some homes. However, we will attempt to make up these samples, within our personnel and equipment restrictions and within fourteen months of the exposure field intensive study.

4.5 TASK 5: PERSONAL, NEIGHBORHOOD, AND SCHOOL SAMPLING

A small pilot study of personal exposure sampling will be conducted during Year 2 for 25 selected students. An ARB-equipped and -operated mobile-monitoring van will perform 2- to 7-day monitoring and sample collection during Years 2 and 3 at selected neighborhood and school sites.

The personal sampling program will include the following major components:

- Preparation of personal sampling equipment. Equipment will consist of passive NO_2 and ozone badges and a battery-operated personal particle sampler with cuts for less than 2.5 μm and 2.5-10 μm. The sampler will be placed in a backpack with the passive badges attached to the straps.
- Selection of children. Children 8 years and older will be selected from homes being sampled during the Exposure Intensive Study, Task 4.
- Training and sample collection. Children will be trained to carry the equipment and to complete the questionnaire and activity diary for the 48-hr sample-collection period.
- Laboratory analysis. The same laboratories and laboratory procedures will be used for the NO_2, ozone, PM mass, endotoxin, and ETS personal samples as will be used for similar samples in other parts of the program.
- Data analysis. After data review and validation, we will describe and display the personal exposure data to illustrate its characteristics. We will then perform comparisons of the personal data with available microenvironmental data and with exposure model estimates.

The ARB has plans to equip several monitoring vans for deployment in various locations of interest in California. The monitoring vans are expected to contain continuous monitors for gaseous and PM agents such as ozone, NO and NO_2, particle scattering, $PM_{2.5}$ and PM mass,

$PM_{2.5}$ OC and $PM_{2.5}$ EC, and $PM_{2.5}$ sulfate and nitrate. The vans are also expected to contain filter samplers for daily $PM_{2.5}$ and PM_{10} mass, ions, and metals concentrations; an additional filter sampling leg could be used to collect samples for PM_{10} endotoxins. After the exposure field intensive study is over, we could also provide samplers for spores and pollens to be installed in the vans. Based on preliminary data analyses, we would select several neighborhoods with a potential of significant health agent impacts and recommend that a sampling van be deployed in those neighborhoods for a period of 2 to 7 days each. We would also identify and recommend several schools in the area for mobile sampling. We assume that ARB would operate the sampling van, perform laboratory analyses, and process and quality-control the sampling-van data.

4.6 TASK 6: DATA ANALYSIS AND EXPOSURE MODELING

4.6.1 Analysis of Central-Site Data

The central site air quality and meteorological measurements will be examined in order to understand conditions at the site; to reconcile the different measurement techniques; to understand the temporal variability of gases and particles; and to understand relationships between gases, particle mass, particle chemical composition, particle size, particle number densities, temperature, and relative humidity in Fresno. Summary statistics, correlation analysis, time-series plots, scatter-plots, and box-whisker plots will be used to understand the central site data. Seasonal, monthly, daily, day-of-week, and diurnal displays of the concentrations will be examined to characterize the temporal variability. All the Group I and Group II agents will be included in this analysis.

4.6.2 Analysis of Neighborhood Data

In the first year of the study, the neighborhoods of all 450 participants will be categorized with respect to probable mobile source activity and vegetation. Land-use, vegetation biomass, and average daily traffic volumes on moderate and heavily traveled roadways (from CALTRANS) will be displayed along with residence and school locations in a Graphic Information System (GIS). This information will be initially used to select homes for the intensive, home-monitoring study and subsequently used as part of the analysis of the neighborhood-scale pollutant data.

In Years 2 and 3, the data collected at neighborhood locations in this study will be used to characterize the within-community variability in outdoor pollutant concentrations. The following data sets will be examined for consistency and analyzed to determine spatial and temporal variability in Fresno outdoor air concentrations:

- Concentration of PM mass, PM chemical components, selected gases, pollens, spores, and endotoxins collected at the central site and four other community-monitoring stations during the two-month CRPAQS winter intensive-sampling program.

- Continuous (hourly) light scattering and integrated ozone, NO_2, and PM concentration data collected outside 96 different homes for one to two weeks during the one-year intensive sampling programs
- Two-week integrated ozone concentrations collected outside homes in the May-September period during the first two years of routine panel studies
- ARB sampling vans' continuous and integrated measurements of outdoor concentrations and particle number density at selected schools and homes in the community.

Exploratory data analysis will be performed. This will include examination of spatial plots of the neighborhood and central-site data. Correlation and regressions will be performed to assess how well the neighborhood-scale concentrations track the central-site concentrations. The magnitude of the bias between specific neighborhood locations and the central-site concentrations will be examined. The high time resolution data, such as the light scattering from nephelometers outside homes, will be compared to the central-site data to assess how the bias varies with time of day and/or day of week. The diurnal dependence will be examined. The extent to which factors, such as proximity to roadways and traffic density, as well as the type and extent of vegetation, explain the variance in concentrations across the city will be assessed. These analyses of covariates are designed to provide submodels for use in the overall exposure model.

The coherence of results for different species will be examined. The consistency in species that are co-emitted from major source types will be evaluated. Comparisons will be made of the spatial patterns and seasonality of the bioaerosols and other pollutants. Finally, we will provide an assessment of which pollutants should be treated as regional (Group I) and neighborhood (Group II) in the model-building exercises.

4.6.3 Analysis of Indoor/Outdoor (I/O) Pollutant Relationships

The data from the indoor and outdoor sampling at participants' homes will be analyzed and categorized to understand how much variation exists in the homes of our study population. The housing questionnaire data will be used in the analysis to determine which characteristics explain differences in I/O ratios and concentrations. Variability in I/O ratios and ventilation characteristics, which will affect all pollutants, will be examined. The relationships between indoor pollutant levels and probable sources, including NO_2 and gas-appliance use; ETS and smoking; pollens and house plants; and $PM_{2.5}$ and smoking, cooking, cleaning, and pets. Representative models will be developed to show how much outside air infiltrates the indoor environment (expressed as I/O ratios for different pollutants and types of structures) and how much indoor sources typically contribute to indoor concentrations. Submodels will be developed from these data that can be applied to all the study participants.

4.6.4 Analysis of Home-Specific Data for Indoor Concentrations of Selected Agents

The Group II and III pollutants will be analyzed to determine which pollutants are primarily indoor contaminants, with little relation to outdoor air (i.e., Group III pollutants). The

indoor measurements of house-dust allergens and ETS data are being collected to use directly in the exposure model, so it will not be necessary to develop models of their concentrations. Nevertheless, statistical summaries of these data will be generated, and the extent to which household characteristics explain the variability of indoor concentrations will be examined.

4.6.5 Analysis of Time-Activity Data for Individual Participants

The time-activity questionnaire data, collected on a daily basis during the panel studies, will be analyzed by day type (weekday versus weekend) and season (summer versus non-summer) for the fractional time spent in the ten to twelve dominant locations. The analysis will primarily consider data collected in the first two years of the study. The means and variance of time-activity within subjects and between subjects will be examined. The time-activity data for each participant will be aggregated into the categories included in the exposure model.

4.6.6 Model Assembly and Testing

Modeling software will be implemented to estimate the daily personal exposures of the study participants to the agents. STI has developed exposure modeling software for cohort exposure assessments where subject specific information is available. STI's existing FORTRAN exposure modeling software will be modified to interface with the subject-specific data available for the Fresno cohort and to incorporate the specific submodels that show the greatest explanatory power. The model will be interfaced with the housing questionnaire data, the time-activity data, the central-site ambient air quality data, the home-specific data for Group III agents, and a library of microenvironmental concentration submodels. The framework will allow alternative submodels to be easily tested.

Testing will be conducted in several stages. In the first stage, the software will be carefully checked to assure the interface with the new database and changes in program logic are working correctly. In a second stage, preliminary sensitivity analyses will be conducted to identify important parameters (or perhaps unimportant parameters). Some model refinements may be made to make better use of the available data, based on these sensitivity results. In the third stage, the model will be tested against the $PM_{2.5}$ and $PM_{2.5-10}$ personal exposure data collected on the Fresno participants. These data are quite limited but will provide an important reality check for the model.

Lastly, the model will be applied for all the study participants to estimate their daily exposures during the first two years of the study. The within-subject and between-subject variability in estimated exposures will be displayed. The variance in exposures from different pollutants and groups of pollutants will be examined and interpreted. Recommendations will be developed for the optimum use and interpretation of these estimates for health analyses.

4.7 TASK 7: QUALITY ASSURANCE

Our quality assurance program will include the following major components:

- Laboratory analysis of additional samples (about 10 percent) to confirm and document field and laboratory blank levels and precision estimates for each agent.
- Calibrations of samplers and monitors at the beginning and end of the exposure field study and of the Fresno Supersite sampling period, including yearly calibrations at the Fresno Supersite.
- Standard Operating Procedures (SOPs). SOPs will be used for field sampling and field and laboratory measurements. In general, we will modify existing SOPs for use in this program. Sample SOPs and a sample home-survey form are included in Appendix B. Additional SOPs that we will modify for use in this program include those we provided for the USC/ARB Children's Health Study (Main et al., 1994a; Main et al., 1994b; and Wright et al., 1994).
- Participation in national allergen programs and interlab comparisons. The person who will read and count pollens and spores will participate in the National Aeroallergen Bureau's PAT program and interlab duplicate readings of slides by Estelle Levetin. The endotoxin analyses will include interlab comparisons (i.e., analysis of split samples) with Don Milton's group.
- Systems audits of the field sampling and monitoring procedures and of the data management system. Procedures to be reviewed will also include filter sample handling, field and laboratory records, and chain-of-custody records and procedures. Knowledgeable staff who are not involved in either the field sampling or the data management activities will perform the systems audits.
- Data processing and validation using standard procedures. Many of these standard procedures will be used in the CRPAQS program for the same or similar measurements.

4.8 TASK 8: DATA MANAGEMENT

Our data management activities will include the following:

- Design and construction of a Microsoft ACCESS database that will directly ingest field and laboratory data and provide the data display and output capabilities needed for the data validation, data analysis, and exposure modeling tasks, as well as for delivery to the heath team.
- Review of routine sample collection at the Fresno Supersite and in the routine two-week panel sampling.
- Regular (at least quarterly) ingest and review of CRPAQS and project data.
- Review of the validation status of the CRPAQS data to confirm that the validation has already been performed. If it has not, the CRPAQS data manager will be notified.
- Validation of project data as it is delivered to the database.

- Delivery of project data to those performing data analysis and exposure modeling and to the health team.

4.9 TASK 9: DOCUMENTATION

As part of the documentation and reporting task, we will prepare and deliver the following:

- Monthly progress reports.
- A refined Protocol describing the activities to be performed during Tasks 2 through 9.
- A draft and final report on the work performed for this project and the project results. The results to be included in these reports will use the data collected during the first two to three years of the study, as described in this proposal and in the refined Protocol.
- Several draft manuscripts prepared for publication in scientific journals.

We will also prepare summary material for presentation at various quarterly and annual meetings to be held jointly with the health team.

4.10 REFERENCES

Main H.H., Chinkin L.R., Schoell B.M., Roberts P.T., and Lurmann F.W. (1994a) Quality assurance plan for microenvironmental ozone measurements in schools as part of the epidemiologic investigation to identify chronic health effects of air pollutants. Version No. 02 prepared for the California Air Resources Board, Sacramento, CA by Sonoma Technology, Inc., Santa Rosa, CA, STI-92372-1424-SOP, August.

Main H.H., Wright D.B., Stoelting M.W., Roberts P.T., Lurmann F.W., and Hering S.V. (1994b) Quality assurance plan for the two-week acid/aerosol sampler as part of the epidemiologic investigation to identify chronic health effects of air pollutants in Southern California. Version No. 3 prepared for the California Air Resources Board, Sacramento, CA by Sonoma Technology, Inc., Santa Rosa, CA and Aerosol Dynamics, Inc., Berkeley, CA, STI-92372-1426-SOP, October.

Wright D.B., Main H.H., and Roberts P.T. (1994) Quality assurance plan for the continuous air quality measurements as part of the epidemiologic investigation to identify chronic health effects of air pollutants. Version No. 2 prepared for the California Air Resources Board, Sacramento, CA by Sonoma Technology, Inc., Santa Rosa, CA, STI-92371-1427-SOP, August.

5. PROJECT MANAGEMENT AND SCHEDULE

5.1 PROJECT MANAGEMENT AND ORGANIZATION

5.1.1 Principal Investigator

The Principal Investigator for the study will be Dr. S. Katharine Hammond. Dr. Hammond is an Associate Professor of Environmental Health Sciences at the School of Public Health, University of California, Berkeley (UCB). She received a master's degree in environmental health sciences from Harvard University and a doctorate in chemistry from Brandeis University. She is a licensed industrial hygienist and is Director of the Industrial Hygiene Program in the School of Public Health.

Dr. Hammond's primary research area is exposure assessment for epidemiologic studies. As indicated by her curriculum vitae, attached in Appendix A, she is extremely well-qualified to lead this particular exposure assessment study because of her combined chemistry and exposure assessment experience and expertise. For example, she developed new methods to sample one of the key airborne chemicals in the study, ETS. She has also developed models to evaluate exposure based on questionnaire data and has validated these models with personal sampling measurements. She designed an exposure assessment strategy for a study of fiberglass workers exposed to a complex mixture of chemicals, among which extensive measurements were made of endotoxin, formaldehyde, and phenolics. She also designed and implemented the exposure assessment for a study of spontaneous abortion among semiconductor workers at fourteen companies across the United States. She has extensive experience measuring ETS in children's homes and developing sampling and laboratory methods for epidemiologic studies. Furthermore, she has collaborated closely with Dr. Ira Tager, Principal Investigator of the health study (Part A), in previous studies.

As Principal Investigator, Dr. Hammond will be responsible for all scientific and financial matters in the study. She will perform day-to-day management of the overall program, oversee all UCB laboratory analyses and personal sampling, manage the subcontractors and consultants, and interface with the project sponsors and the health study investigators.

5.1.2 Project Organization and Communication

The organization of the project team is shown schematically in **Figure 5-1**. This organization diagram shows that the Principal Investigator will be assisted by Co-Investigators, laboratory technicians, field technicians, data analysts, outside consultants, and graduate students. Resumes of key personnel are included in Appendix A. The roles of the principal supporting personnel on the project are as follows:

- **Dr. Ira Tager** will serve as the UCB Principal Investigator for the epidemiologic component of the project as well as the investigator with primary responsibility for

coordination and integration of Parts A and B of the project. Dr. Tager has extensive experience in the design, implementation, and analysis of longitudinal studies of chronic respiratory disease in children, including air pollution-related studies.

- **Dr. Kathleen Mortimer**, UCB, will serve as the Study Director for the epidemiologic component of the project and, as such, will be responsible for the implementation, execution, and oversight of all activities related to data collection, data processing, and data management in Part A of the study.
- **Dr. Janet Macher**, an air pollution research specialist in the CDHS Indoor Air Quality Section, will be a Co-Principal Investigator of the exposure study and will oversee all of the biological sampling, laboratory analysis of biological components, and use of the biological agent data in the exposure and health analyses.
- **Dr. Paul Roberts** is Executive Vice President and Manager of Regional Studies at STI. He will be a Co-Principal Investigator with responsibilities for exposure field measurements, exposure data management, and overall quality assurance (as the project quality assurance officer).
- **Mr. Fred Lurmann** is President and Manager of Exposure Assessment Studies at STI. He will be a Co-Principal Investigator responsible for exposure data analysis and modeling.
- **Dr. Steven D. Colome** is an adjunct professor at the UCLA School of Public Health. His role in the project will be as a consultant on personal and indoor sampling methodologies.
- **Dr. Beth Wittig** is an air quality engineer at STI and will coordinate the exposure field measurements in Fresno.
- **Mr. Charles Perrino** is the Laboratory Manager at the UCB School of Public Health Laboratory and will manage the day-to-day laboratory-related activities on the project.

As indicated in Figure 5-1, the management responsibilities will be assigned by program element. Dr. Hammond has responsibility for the laboratory operations and personal sampling. Dr. Roberts has responsibility for the field measurements and data management. Mr. Lurmann is responsible for the exposure data analysis and modeling. Also, to assure that all of the proposed work is performed properly, we have assigned leaders to each major task, as shown in **Table 5-1**. Dr. Hammond will lead Tasks 1, 3, 5, and 9; Dr. Roberts will lead Tasks 2, 4, 7, and 8; and Mr. Lurmann will lead Task 6.

The keys to good project management are planning, communication, and attention to detail. Dr. Hammond and the Co-Investigators are skilled at project planning and tracking. The project team members, with the exception of consultants, are located in the San Francisco Bay Area and can easily gather in Berkeley or Petaluma for project meetings. We plan to have quarterly and annual project meetings with the project staff (including Drs. Tager and Mortimer) to formally discuss progress and findings. The annual meeting will include the project advisors (see Section 5.1.3 below). The project team members will maintain close contact by telephone, e-mail, and fax on a weekly or daily basis (depending on the level of

project activity). Frequent and inclusive communications, anticipation of potential problems, clear designation of assignments and responsibility, and follow-up on action lists will be used to keep the project on the proper track. The ARB-designated project monitor will be kept informed and generally included in conference calls, e-mails, and project meetings. Wherever possible, the management team will attempt to identify and solve problems when they are small rather than wait until they may become more difficult to resolve.

Table 5-1. Task leader for the proposed exposure study.

Task	Task Leader
Task 1 - Protocol Refinement	Dr. K. Hammond
Task 2 - Augmentation of Central and Neighborhood Site Measurements during CRPAQS	Dr. P. Roberts
Task 3 - Routine Microenvironmental Measurements	Dr. K. Hammond
Task 4 - Exposure Intensive Measurements	Dr. P. Roberts
Task 5 - Personal, Neighborhood, And School Sampling	Dr. K. Hammond
Task 6 - Data Analysis And Exposure Modeling	Mr. F. Lurmann
Task 7 - Quality Assurance	Dr. P. Roberts
Task 8 - Data Management	Dr. P. Roberts
Task 9 - Documentation	Dr. K. Hammond

5.1.3 External Advisory Panel

To further ensure the highest standard of scientific investigation, an External Advisory Panel comprised of distinguished researchers has been assembled for this project (Parts A and B). The panel includes Dr. Frank Speizer (Harvard University), Dr. David Bates (University of British Columbia), Dr. Raymond Neutra (California Department of Health Services), Dr. Mark Segal (University of California, San Francisco), Dr. Leanne Sheppard (University of Washington, Seattle), Dr. Lance Wallace (U.S. Environmental Protection Agency), Dr. Judith Chow (Desert Research Institute, Reno, NV), and Dr. Suresh Moolgavkar (Fred Hutchinson Cancer Center, Seattle WA). The panel will meet annually, and their advice will be sought throughout the study period.

5.2 SCHEDULE

The schedules for Parts A and B of the study have been coordinated. Assuming the first panel study begins in August 2000, Part B should begin on or before July 1, 2000. As indicated in **Figure 5-2**, the study has been planned as a five-year project beginning

July 1, 2000, with the majority of work performed in the first four years. The principal elements of the project schedule are as follows:

- The Protocol refinements will primarily be made in the first year.
- Quarterly and annual project meetings will occur over the entire five-year period when more refinements are possible.
- The central-site monitoring will be enhanced at the beginning of the project (i.e., as soon as we can obtain and install the equipment) and will be operated for 51 months. The samples collected during the first two years at the central site will be subjected to laboratory analysis. The remaining samples will be collected and archived for future analysis.
- Special monitoring will be performed at other community monitoring sites during the CRPAQS winter intensive study, December 1, 2000 to January 31, 2001.
- Routine monitoring will be initiated with the first panel study and will continue for 51 months. The laboratory analyses will focus primarily on the samples collected during the first two years of routine monitoring. The remaining samples will be collected and archived for future analysis.
- Preparation for the intensive exposure monitoring will be performed in the fall of 2000 and winter of 2001. The intensive home sampling will begin in May 2001 and extend through June 2002.
- The personal sampling and ARB-van neighborhood and school monitoring will occur in the same time frame as the intensive exposure monitoring at homes.
- Data analysis in support of neighborhood classification and home selection for intensive monitoring will occur in the first year. Subsequent data analysis and modeling will be performed from January 2002 to June 2003.
- Data management, quality assurance, and documentation will occur throughout the project. Versions of the project database will be delivered annually; the final database will be delivered in the fifth year of the study.
- A draft final report will be prepared in Years 3 and 4; it will be delivered in April 2004.
- A final report that incorporates comments on the draft report will be delivered in September 2004.
- The project team will continue to interact with the health team in Year 5 to assist with analysis and interpretation of the data.

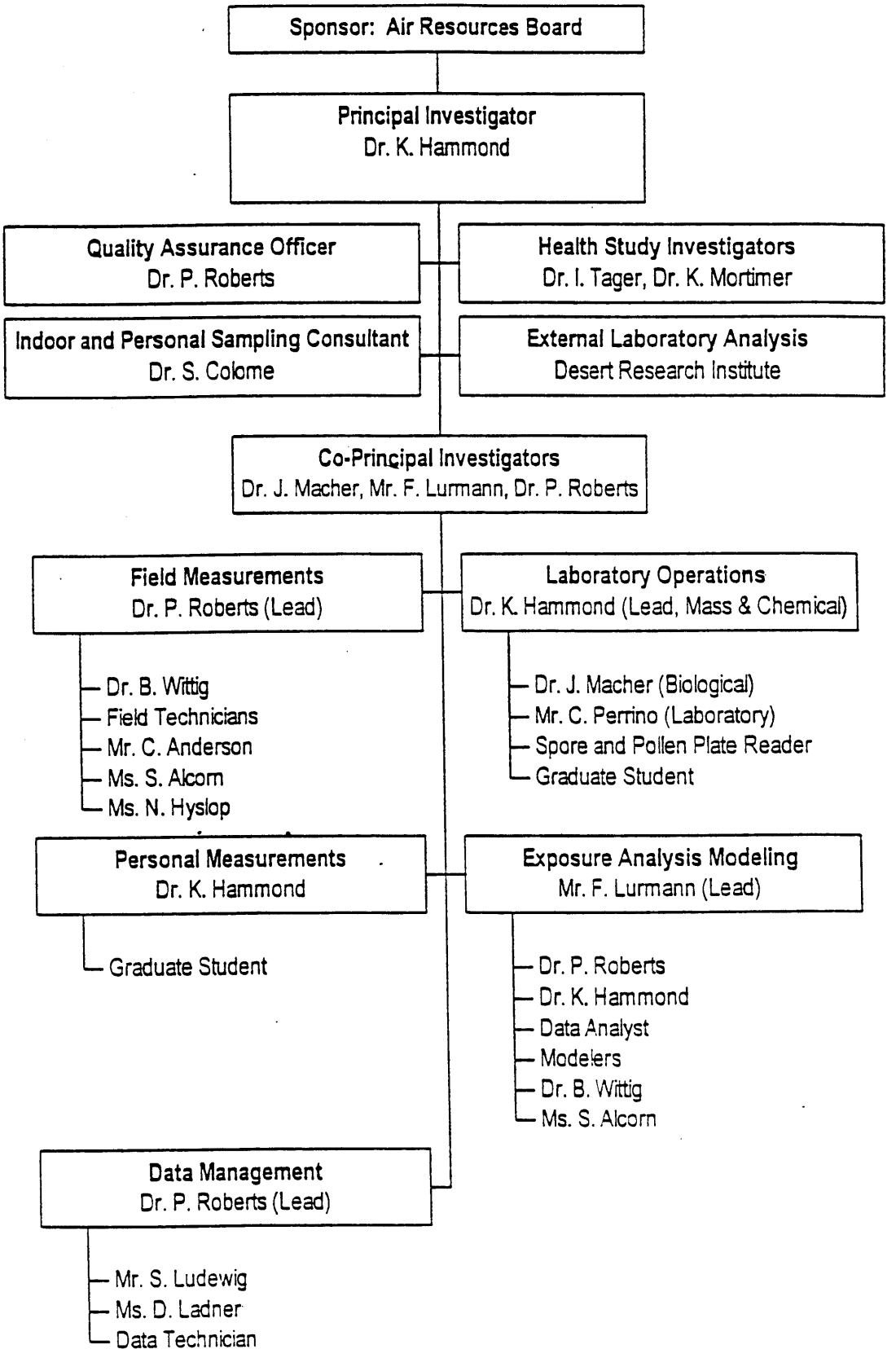

Figure 5-1. Project organization schematic.

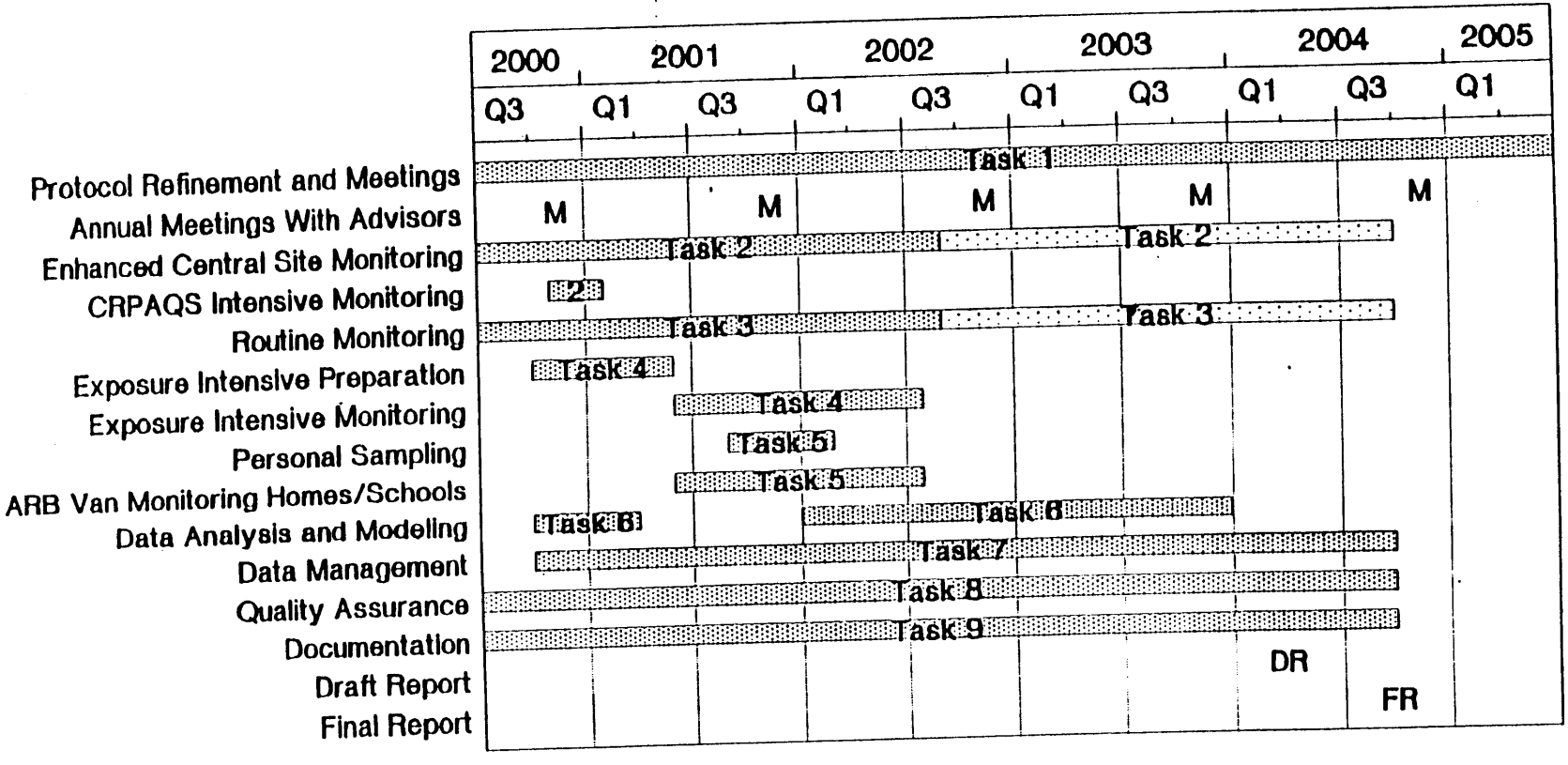

Figure 5-2. Project Schedule.

APPENDIX A

RESUMES

UNIVERSITY OF CALIFORNIA, BERKELEY
SCHOOL OF PUBLIC HEALTH
S. Katharine Hammond, Ph.D., CIH
Charles Perrino

CALIFORNIA DEPARTMENT OF HEALTH SERVICES
Janet M. Macher, Sc.D.

SONOMA TECHNOLOGY, INC.
Frederick W. Lurmann
Paul T. Roberts, Ph.D.
Ann E. (Beth) Wittig, Ph.D.

UNIVERSITY OF CALIFORNIA, LOS ANGELES
SCHOOL OF PUBLIC HEALTH
Steven D. Colome, Sc.D.

CURRICULUM VITAE

S. Katharine Hammond, Ph.D., CIH

September, 1999

Position: Current

Associate Professor of Public Health
Environmental Health Sciences Division
140 Earl Warren Hall
School of Public Health
University of California
Berkeley, California 94720

e-mail: hammondk@uclink4.berkeley.edu
Phone: (510) 643-0289
FAX: (510) 642-5815

Education

Oberlin College	B.A.	1971	Chemistry
Brandeis University	Ph.D.	1976	Chemistry
Harvard School of Public Health	M.S.	1981	Environmental Health Sciences

Postdoctoral Training

1980-1981 Harvard School of Public Health in Environmental Health Sciences
Award: National Institute for Occupational Safety and Health
Postdoctoral Training Fellowship

Licensure:

Certified Industrial Hygienist Specialty: Comprehensive Practice
1984 American Board of Industrial Hygiene #2876

Academic Appointments

1994-present Associate Professor of Environmental Health Sciences
Director, Industrial Hygiene Program
School of Public Health
University of California, Berkeley, California

1993-1994 Director, Environmental Health Division
Department of Family and Community Medicine
University of Massachusetts Medical Center

1989-1994 Associate Professor of Family and Community Medicine and of Pharmacology
(tenured in April, 1993)
University of Massachusetts Medical Center, Worcester, Massachusetts

1985-1989 Assistant Professor of Family and Community Medicine
Environmental Health Sciences Program
University of Massachusetts Medical Center, Worcester, Massachusetts

1985-present Visiting Lecturer on Industrial Hygiene
Harvard School of Public Health, Boston, Massachusetts

1981-1984 Research Associate in Industrial Hygiene
Harvard School of Public Health, Boston, Massachusetts

1976-1980 Assistant Professor of Chemistry
Wheaton College Norton, Massachusetts

1975-1976 Instructor of Science, College of Basic Studies
Boston University Boston, Massachusetts

1974 Instructor of Inorganic Chemistry
Regis College Weston, Massachusetts

TEACHING EXPERIENCE

COURSES TAUGHT

Regis College (1974)
Advanced Inorganic Chemistry
Boston University (1975 - 1976)
Freshman Science (Physics, Chemistry, Meteorology, and Astronomy)
Wheaton College (1976 - 1980)
Chemistry and Our Environment — Advanced Inorganic Chemistry
Analytical Chemistry — Instrumental Analysis
Senior Chemistry Seminar (special topics: Group Theory, Bioinorganic Chemistry, Toxicology)

Harvard University (1981 - 1994)
Identification and Measurement of Air Contaminants jointly taught course (1981 - 1985)
Fundamentals of Industrial Hygiene (short course) various lectures & laboratories (1981 -1994)
Environmental Contaminants, Harvard Extension School, 1990; 1991
Environmental Management, Harvard Extension School, 1992

University of Massachusetts Medical School, Worcester (1985 - 1994)
Environmental Health Practices 1989 - 1994
Occupational and Environmental Medicine (industrial hygiene section) 1992; 1994
Community Medicine 1st Yr Clerkship preceptor 1990 - 1994
Epidemiology and Preventive Medicine (section leader) 1986
Research Seminar in Environmental Health (jointly with T.J. Smith)
- Reproductive Hazards in the Workplace, 1987
- Environmental Carcinogens, 1989
- Modeling Exposures, 1991 - 1992

Introduction to Industrial Hygiene: Preventing Workplace Disease (1 week short course)

University of California School of Public Health, Berkeley (1994 - present)
Characterization of Airborne Chemicals (PH 267b)
Exposure Assessment and Control (PH270a)
Environmental and Occupational Health Issues in the Semiconductor Industry (PH298)
EHS Seminar for Doctoral Students (PH293)
EHS Seminar for MPH Students (PH292)
Freshman Seminar: The Environment: Have We Made a Difference? (PH39)

Other
Occupational Epidemiology, a short course offered for certification maintenance at the American Industrial Hygiene Conference 1989, St. Louis, MO and 1991 Salt Lake City, UT

Center for Occupational and Environmental Health, continuing education short courses
- Fundamentals of Industrial Hygiene (1997 - present):
- Review of Industrial Hygiene (1998)

Workshop on Advanced Methodologies for Occupational and EnvironmentalHealth Studies, course taught in Shun Yi, China, as part of Fogarty Grant, jointly sponsored by UCBerkeley and the Chinese Academy of Preventive Medicine May 26 – June 3, 1998

TEACHING EXPERIENCE (continued)

RESEARCH SUPERVISION

Wheaton College (1976 - 1980)
Undergraduate research, several chemistry majors
Brown University (1979)
Doctoral student dissertation committee (outside examiner)
Harvard School of Public Health (1981 - 1994)
Masters level graduate student research projects in industrial hygiene
Doctoral student research and dissertation committees
Martin Cohen (1987 - 1989)
Michael Walters (1990 - 1993)
Fred Chang (1993 - 1994)
University of Massachusetts Medical School (1985 - 1994)
Medical Resident, research for MPH degree
Jacalyn Coghlin-Strom, M.D. (1985 - 1988)
Postdoctoral Fellows
Elaina Kenyon (1989 - 1991)
Sassan Abdollahzadeh (1992 - 1994)
Medical student research
Marcella Bradway (1991)
Graduate student, Clark University
JoAnne Shatkin (1990 - 1991)
Undergraduate student research projects:
Worcester Polytechnic Institute
Susan Hepworth (1987 - 1988)
Clark University Senior Honors project
Alison Draper (1991 - 1992)
Clark University Undergraduate students
Alison Draper (1989 - 1992)
So Yoon Jang (1992 - 1994)
Yale University
Reader for Doctoral Dissertation, Terrance O'Connor (1993)
University of Massachusetts, Lowell (1993 - 1994)
Doctoral student research and dissertation committee
Arthur Lu (1993 - 1994), Work Environment Department

University of California School of Public Health, Berkeley (1994 - present)
Undergraduate research advisor
Christine Quan 1997-1998
Masters student research advisor
Nicole Reilly 1995-1996
Shao-wen Liaw 1996-1998
Doctoral student research advisor
Melissa Gonzales (1994-1998) co-chair
Michael Apte (1994-1997)
Gita Murthy (1995 - 1999) co-chair
Kathleen Vork (1995 - present)
Neil Klepeis (1997 -1998)
Michael Wilson (1998 – present)
Linwei Tian (1998- present)

Other Professional Activities

Scientific Societies

American Chemical Society
American Industrial Hygiene Association
American Conference of Governmental Industrial Hygienists
American Public Health Association
American Association for the Advancement of Science
International Society of Exposure Analysis

Service on Scientific Advisory Committees

1980 National Science Foundation Peer Review Committee
1982-84 American Public Health Association, Occupational Health and Safety Section Task Force on Victim Compensation
1987-93 National Institute of Health Study Sections: ad hoc reviewer on four occasions (Occupational Safety and Health; and, Environmental Health Sciences)
1987-89 New Technologies Safety & Health Institute Steering Committee
1988-present American Industrial Hygiene Assn: Occupational Epidemiology Committee Secretary, 1989-1990; Vice Chair, 1990-1991; Chair, 1991-1992; Past Chair, 1992-1993
1988-94 Harriet Hardy Institute, Board of Directors
1990-present Consultant to the Science Advisory Board of the United States Environmental Protection Agency (participated in the review of the environmental tobacco smoke documents, 1990-1992, that resulted in the official publication of *Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders*)
1992-97 American Conference of Governmental Industrial Hygienists Chemical Substances Threshold Limit Value Committee
1993-96 International Society of Exposure Analysis, Councilor
1996-98 International Society of Exposure Analysis, Treasurer
1994-97 National Institute of Environmental Health Sciences *ad hoc* reviewer for Site Visit of Center Grants
1994-97 National Cancer Institute, Acrylonitrile Advisory Panel
1994-present National Research Council, National Academy of Sciences Committee on Health Effects of Waste Incineration
1995-96 Institute of Medicine, National Academy of Sciences Committee on the Health Effects in Vietnam Veterans of Exposure to Herbicides, First Biannual Update
1995 National Cancer Institute/National Institute for Occupational Safety and Health, *ad hoc* reviewer for grants
1996 American Industrial Hygiene Association--Northern California Section Strategy Committee
1996-present Institute of Medicine, National Academy of Sciences Committee on the Assessment of Wartime Exposure to Herbicides in Vietnam
1999-present Advisory Committee for Energy-Related Epidemiologic Research of the Centers for Disease Control and Prevention

Invited speaker at conferences and symposia

Environmental Protection Agency/Air Pollution Control Association Symposium on Measurement of Toxic Air Pollutants, Raleigh, North Carolina, May 1986
International Workshop on Exposure Assessment for Epidemiology and Hazard Control, Woods Hole, Massachusetts April, 1988
Hazard Surveillance, Amherst, Massachusetts 1993
Semiconductor Safety Institute, Scottsdale, Arizona, April, 1993
Workshop on Exposure Assessment sponsored by the Republic of China National Academy of Sciences and the Republic of China Environmental Protection Agency. Taipei, Taiwan May 31, 1993.
EPRI Electromagnetic Field Seminar: Focus on Research Santa Clara, California, March, 1994
Workshop on Developing an Exposure Assessment Program for Occupational Epidemiologic Studies. National Cancer Institute and National Institute for Occupational Safety and Health. Virginia, March 28-29, 1994
International Symposium on Health Hazards of Glycol Ethers. Abbaye de Pont-a-Mousson, Nancy, France April 19-21, 1994.
Exposure Assessment Workshop, Monash University, Melbourne, Australia August 22, 1995.
Workshop on Gender Differences in Exposures and Susceptibilities. Institute of Medicine, National Academy of Sciences, May 21, 1997, Washington, D.C.
Workshop on Assessment of Exposure to Environmental Tobacco Smoke, Johns Hopkins University, Baltimore, Maryland. September, 1997.
Workshop on Linking Environmental Agents and Autoimmune Disease. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, September 2, 1998.

Manuscript reviewer

Science
American Industrial Hygiene Association Journal
Air Pollution Control Association Proceedings
American Journal of Public Health
Environmental Science and Technology
Atmospheric Environment
Journal of the American Medical Association
Indoor Air
Oncology Review
Applied Occupational and Environmental Hygiene
Cancer Research
American Journal of Epidemiology
American Journal of Industrial Medicine
Tobacco Control
Journal of Exposure Analysis and Environmental Epidemiology
Cancer Epidemiology, Biomarkers, and Prevention
Heath Education and Behavior
Risk Analysis

Service at University of California, Berkeley

University of California, President's Panel on Environmental Health and Safety at the National Laboratories, 1994-1997
School of Public Health, Faculty Council, secretary, 1997-1998
Safety Committee, School of Public Health, 1995-1998
Research Committee, School of Public Health-- 1995-1998
Multicultural Committee, School of Public Health--1996—1998; Chair, 1997-99
Faculty Diversity Subcommittee, Chair, 1996—1997
Admission Committee, Environmental Health Sciences Division
Local Area Network Computing Committee, Environmental Health Sciences
Center for Occupational and Environmental Health, Executive Committee 1994-present
UCOP, Toxic Substances Research and Training Program, Executive Committee 1997-present
School of Public Health, Curriculum committee, 1998-present
Chair, Environmental Health Sciences Graduate Group, 1998-present
NIOSH Educational Research and Training Center, Acting Chair, 1999-present
DrPH Management Committee, 1990-present

BIBLIOGRAPHY

Peer Reviewed Journal Articles

1. Ackermann, M.N., Hallmark, M.R., Hammond, S.K., Roe, A. N. Alkyldiazenes. Gas-phase and solution chemistry. Physical and chemical properties of methyldiazene. *Inorganic Chemistry.* **11**:3076-3082, 1972.
2. Hammond (Gilbert), K. and Kustin, K. Kinetics and mechanism of molybdate and tungstate complex formation with catechol derivatives. *Journal of the American Chemical Society.* **98**:5502-5512, 1976.
3. Hammond (Gilbert), K. Kustin, K. and McLeod, G.C. Gel filtration analysis of vanadium in *Ascidia nigra* blood cell lysate. *Journal of Cellular Physiology.* **93**:309-311, 1977.
4. Smith, T. J., Hammond, S. K. Laidlaw, F., Fine, S. Respiratory exposures associated with silicon carbide production: estimation of cumulative exposure for an epidemiological study. *British Journal of Industrial Medicine.* **41**: 109-115, 1984.
5. Peters, J. M., Smith, T. J., Bernstein, L., Wright, W.E., Hammond, S.K. Pulmonary effects of exposures in silicon carbide manufacturing. *British Journal of Industrial Medicine.* **41**:109-115, 1984.
6. Hammond, S.K., Leaderer, B.P., Roche, A.C., and Schenker, M. Collection and analysis of nicotine as a marker for environmental tobacco smoke. *Atmospheric Environment.* **21**:457-461, 1987.
7. Hammond, S.K., Smith, T.J., Ellenbecker, M.J. Determination of occupational exposure to fabric brightener chemicals by HPLC. *American Industrial Hygiene Association Journal.* **48**:117-121, 1987.
8. Hallock, M.F., Smith, T.J., Hammond, S.K., Beck, B.D., and Brain, J.D. A new technique for collecting ambient diesel particulate for bioassays. *American Industrial Hygiene Association Journal.* **48**: 487-493, 1987.
9. Garshick, E., Schenker, M.B., Muinoz, A., Segal, M., Smith, T.J., Woskie, S.R., Hammond, S.K., Speizer, F.E. A case-control study of lung cancer and diesel exhaust exposure in railroad workers. *American Review of Respiratory Diseases.* **135**:1242-1248, 1987.
10. Hammond, S.K., and Leaderer, B.P. A diffusion monitor to measure exposure to passive smoking. *Environmental Science and Technology.* **21**:494-497, 1987.
11. Woskie, S.R., Smith, T.J., Hammond, S.K., Schenker, M.B., Garshick, E. Speizer, F.E. Estimation of the diesel exhaust exposures of railroad workers: I. Current exposures. *American Journal of Industrial Medicine.* **13**: 381-394, 1988.
12. Woskie, S.R., Smith. T.J., Hammond, S.K., Schenker, M.B., Garshick, E., Speizer, F.E. Estimation of the diesel exhaust exposures of railroad workers: II. National and Historical. *American Journal of Industrial Medicine.* **13**: 395-404, 1988.
13. Garshick, E., Schenker, M.B., Munoz, A., Segal, M., Smith, T.J., Woskie, S.R., Hammond, S.K., Speizer, F.E. A retrospective cohort study of lung cancer and diesel exhaust exposure in railroad workers. *American Review of Respiratory Diseases.* **137**: 820-825, 1988.
14. Hammond, S.K., Smith, T.J., Woskie, S.R., Leaderer, B.P., and Bettinger, N. Markers of exposure to diesel exhaust and cigarette smoke in railroad workers. *American Industrial Hygiene Association Journal.* **49**:516-522, 1988.
15. Mattson, M.E., Boyd, G., Byar, D., Brown, C., Callahan, J.F., Cullen, J.W., Greenblatt, J., Haley, N., Hammond, S.K., Lewtas, J., Reeves, W. Passive Smoking on Commercial Airline Flights. *Journal of the American Medical Association,* **261**: 867-872, 1989.
16. Lofroth, G., Burton, R., Forehand, L., Hammond, S.K., Seila, R., Zweidinger, R., and Lewtas, J. Characterization of environmental tobacco smoke. *Environmental Science and Technology*, **23**: 610-614, 1989.

17. Osterman, J.W., Greaves, I.A., Smith, T.J., Hammond, S.K., Robbins, J.M., Theriault, G. Respiratory symptoms associated with low level sulfur dioxide exposure in silicon carbide production workers. *British Journal of Industrial Medicine*, **46**: 629-635, 1989.
18. Osterman, J.W., Greaves, I.A., Smith, T.J., Hammond, S.K., Robbins, J.M., Theriault, G. Work related decrement in pulmonary function in silicon carbide production workers. *British Journal of Industrial Medicine*, **46**: 708-716, 1989.
19. Henderson, R.W., Reid, H.F., Morris, R., Wang, Ou-Li, Hu, P.C., Helms, R.W., Forehand, L., Mumford, J., Lewtas, J., Haley, N.J., and Hammond, S.K. Home air nicotine levels and urinary cotinine excretion in preschool children. *American Review of Respiratory Diseases*, **140**: 197-201, 1989.
20. Coghlin, J., Hammond, S.K., and Gann, P. Development of epidemiologic tools for measuring environmental tobacco smoke exposure. *American Journal of Epidemiology*, **130**:696-704, 1989.
21. Woskie, S.R., Hammond, S.K., and Smith, T.J. Current nitrogen dioxide exposures among railroad workers. *American Industrial Hygiene Association Journal*, **50**: 346-353, 1989.
22. Leaderer, B. P., Boone, P.M., White, J.B., and Hammond, S.K. Total particle, sulfate, and acid aerosol emissions from kerosene space heaters. *Environmental Science and Technology*, **24**: 908-912, 1990.
23. Caka, F.M., Eatough, D.J., Lewis, E.A., Tang, H., Hammond, S.K., Leaderer, B.P., Koutrakis, P., Spengler, J.D., Fasano, A., McCarthy, J., Ogden, M.W., and Lewtas, J. An Intercomparison of sampling techniques for nicotine in indoor environments. *Environmental Science and Technology*, **24**:1196-1203, 1990.
24. Kelsey, K.T., Smith, T.J., Hammond, S.K., Letz, R., and Little, J.B. Sister chromatid exchanges in lymphocytes from styrene-exposed boat builders. *Mutation Research*, 241:215-221, 1990.
25. Quinn, M.M., Wegman, D.H., Greaves, I.A., Hammond, S.K., Ellenbecker, M.J., Spark, R.F., and Smith, E.R. Investigation of reports of sexual dysfunction among male chemical workers manufacturing stilbene derivatives. *American Journal of Industrial Medicine*, **18**:55-68, 1990.
26. Day, Billy W., Skipper, Paul L., Wishnok, John S., Coghlin-Strom, Jacalyn, Hammond, S. Katharine, Gann, Peter, and Tannenbaum, Steven R. Identification of an in Vivo chrysene diol epoxide adduct in human hemoglobin. *Chemical Research in Toxicology*, **3**:340-343, 1990.
27. Cohen, Martin A., Ryan, P. Barry, Yanagisawa, Yukio, and Hammond, S. Katharine. The Validation of a Passive Sampler for Indoor and Outdoor Concentrations of Volatile Organic Compounds. *Journal of Air and Waste Management Association*, **40**: 993-997, 1990.
28. Vaughan, W. M., and Hammond, S.K. Impact of "Designated smoking area" policy on nicotine vapor and particle concentrations in a modern office building. *Journal of Air and Waste Management Association*, **40**:1012-1017, 1990.
29. Coghlin, J. Gann, P., Hammond, S.K., Paul, M., Taghizadeh, K., Skipper, P., and Tannenbaum, S.R. 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen in utero. *Journal of the National Cancer Institute*, **83**: 274-280, 1991.
30. Leaderer, B.P. and Hammond, S.K. Evaluation of vapor-phase nicotine and respirable suspended particle mass as markers for environmental tobacco smoke. *Environmental Science and Technology*, **25**:770-777, 1991.
31. Kado, N.Y., McCurdy, S., Tesluk, S.J., Hammond, S.K., Hsieh, D.P.H., Jones, J. and Schenker, M.B. Measuring personal exposure to airborne mutagens and nicotine in environmental tobacco smoke. *Mutation Research*, **261**:75-82, 1991.
32. Smith, T.J., Hammond, S.K., Hallock, M., and Woskie, S.R. Exposure assessment for epidemiology: Characteristics of exposure. *Applied Occupational and Environmental Hygiene*, **6**:441-447, 1991.

33. Hammond, S.K., Shatkin, J. and Leaderer, B.P. Determination of the mass extractable in organic solvents by evaporative light scattering detection. *Applied Occupational and Environmental Hygiene*, 7:49-54, 1992.
34. Schenker, M.B., Kado, N.Y., Hammond, S.K., Samuels, S.J., Woskie, S.R., and Smith, T.J. Urinary mutagenic activity in workers exposed to diesel exhaust. *Environmental Research*, **57**:133-148, 1992.
35. Nagda, Niren L., Koontz, Michael D., Konheim, Arnold G., and Hammond, S. Katharine. Measurement of cabin air quality aboard commercial airliners. *Atmospheric Environment*, **26A**:2203-2210, 1992.
36. Hammond, S.K., Coghlin, J., Gann, P.H., Paul, M. Taghizadeh, K. Skipper, P.L., and Tannenbaum, S.R. Relationship between environmental tobacco smoke exposure and carcinogen-hemoglobin adduct levels in nonsmokers. *Journal of the National Cancer Institute*, **85**:474-478, 1993.
37. Marbury, M.C., Hammond, S.K., and Haley, N.J. Measuring exposure to environmental tobacco smoke in studies of acute health effects. *American Journal of Epidemiology*, **137**:1089-1097, 1993.
38. Kenyon, E.M., Hammond, S.K., Shatkin, J. Woskie, S.R., Hallock, M.F., and Smith, T.J. Ethanolamine exposures in machining fluids. *Applied Occupational and Environmental Hygiene*, **8**:655-661, 1993.
39. Hallock, M.F., Hammond, S. K., Kenyon, E., Smith, T.J., and Smith, E. Assessment of task and peak exposures to solvents in the microelectronics fabrication industry. *Applied Occupational and Environmental Hygiene*, **8**:945-954, 1993.
40. Hammond, S.K., Smith, T.J., Woskie, S.R., Braun, A.G., Liber, H., Lafleur, A., Garshick, E., Schenker, M.B., and Speizer, F. Railroad diesel exhaust: concentration and mutagenicity. *Applied Occupational and Environmental Hygiene*, **8**:955-963, 1993.
41. Smith, T.J., Hammond, S.K., and Wong, O. Health effects of gasoline exposure: I. Exposure assessment for U.S. distribution workers. *Environmental Health Perspectives*, **101** (Suppl. 6): 13-21, 1993.

Above were all published and submitted as part of my original appointment and tenure considerations

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The following were in press:

42. Woskie, S.R., Smith, T.J., Hallock, M.F., Hammond, S.K., Rosenthal, F., Eisen, E.A., Kriebel, D., and Greaves, I. Size selective pulmonary dose indices for coolant fluid aerosols in machining and grinding operations in the automobile manufacturing industry. *American Industrial Hygiene Association Journal*, **55**:20-29, 1994.
43. Hallock, M.F., Smith, T.J., Woskie, S.R., and Hammond, S.K. Estimation of historical exposures to machining fluids in the automotive industry. *American Journal of Industrial Medicine*, **26**:621-634, 1994.

The following had been submitted for publication:

44. Emmons, K.M., Hammond, S.K., and Abrams, D.B. The Impact of smoking cessation on nonsmokers' exposure to environmental tobacco smoke. *Health Psychology*, **13**:516-520, 1994.
45. Paul, M.E., Hammond, S.K., Abdollahzadeh, S. Assessment of exposure to low-frequency magnetic fields in a neonatal intensive care nursery. *Bioelectromagnetics*: **15**:519-529, 1994.
46. Milton, DK, Walters, MD, Hammond, SK, Evans, JS. and Worker exposure to endotoxins and other air contaminants in a fiberglass insulation manufacturing facility. *American Industrial Hygiene Association Journal* **57**:889-896, 1996.

The following are entirely new:

47. Woskie, S.R., Smith, T.J., Hammond, S.K., and Hallock, M.F. Factors affecting worker exposures to metal-working fluids during automotive component manufacturing. *Applied Occupational and Environmental Hygiene*, **9**:612-621, 1994
48. Abrams, D.B., for the Working Well Research Group. Cancer control at the workplace: The Working Well trial. *Preventive Medicine* **23**:15-27, 1994.
49. Maurer, K.F., Cullen, M.R., Erdil, M., Dupuy, C.J., Jung, B.C., Hadler, J.L., Castler, B. Hammond, S.K. Controlling Lead Toxicity in Bridge Workers--Connecticut, 1991-1994. *Morbidity and Mortality Weekly Report*. **44**:76-79, 1995.
50. Heimendinger, J. et al., for the Working Well Research Group. The Working Well Trial: Baseline dietary and smoking behaviors of employees and related worksite characteristics. *Preventive Medicine*. **24**:180-193, 1995.
51. Sorensen, G., Himmelstein, J., Hunt, M.K., Youngstrom, R., Hebert, J., Hammond, S.K., Palombo, R., Stoddard, A., and Ockene, J.K. A Model for worksite cancer prevention: Integration of health protection and health promotion in the WellWorks project. *American Journal of Health Promotion*, **10**:55-62, 1995.
52. Schenker, M.B., Gold, E.B., Beaumont, J., Eskenazi, B.E., Hammond, S.K., Lasley, B.L., McCurdy, S., Samuels, S., Saiki, C.L., Swan, S.H. Association of spontaneous abortion and other reproductive effects with work in the semiconductor industry. *American Journal of Industrial Medicine*, **28**:639-659, 1995.
53. Hammond, S.K., Hines, C.J., Hallock, M.F., Woskie, S.R., Abdollahzadeh, S., Iden, C.R., Ramsey, R., Anson, E., Schenker, M.B. The Tiered exposure-assessment strategy in the semiconductor health study. *American Journal of Industrial Medicine*, **28**:661-680, 1995.
54. Hallock, M.F., Hammond, S.K., Hines, C.J., Woskie, S.R., Schenker, M.B. Patterns of chemical use and exposure control in the semiconductor health study. *American Journal of Industrial Medicine*, **28**:681-697, 1995.
55. Woskie, S.R., Hammond, S.K., Hines, C.J., Hallock, M.F., Schenker, M.B. Algorithms for estimating personal exposures to chemical agents in the semiconductor health study. *American Journal of Industrial Medicine*, **28**:699-711, 1995.
56. Hines, C.J., Selvin, S., Samuels, S.J., Hammond, S.K., Woskie, S.R., Hallock, M.F., and Schenker, M.B. Hierarchical cluster analysis for exposure assessment of workers in the semiconductor industry. *American Journal of Industrial Medicine*, **28**:713-722, 1995.
57. Abdollahzadeh, S., Hammond, S.K., Schenker, M.B. A Model for assessing occupational exposure to low frequency magnetic fields in fabrication rooms in the semiconductor health study. *American Journal of Industrial Medicine*, **28**: 723-734, 1995.
58. Beaumont, J.J., Swan, S.H., Hammond, S.K., Samuels, S., Green, R.S., Hallock, M.F., Dominguez, C., Boyd, P. Schenker, M.B. Historical cohort investigation of spontaneous abortion in the semiconductor health study: Methods and analyses of risk in fabrication overall and in fabrication work groups. *American Journal of Industrial Medicine*, **28**:735-750, 1995.
59. Swan, S.H., Beaumont, J. Hammond, S.K., Von Behren, J., Green, R.S., Hallock, M., Woskie, S.R., Hines, C.J., Schenker, M.B. Historical cohort study of spontaneous abortion among fabrication workers in the semiconductor health study: Agent-specific analysis. *American Journal of Industrial Medicine*, **28**:751-769, 1995.
60. Gold, E.B., Eskenazi, B., Hammond, S.K., Lasley, B., Samuels, S., Rasor, M.O'N., Hines, C.J., Overstreet, J. Schenker, M.B. Prospectively assessed menstrual cycle characteristics in female wafer-fabrication and nonfabrication semiconductor employees. *American Journal of Industrial Medicine*, **28**: 799-815, 1995.
61. Eskenazi, B., Gold, E.B., Samuels, S., Wight, S., Lasley, B.L., Hammond, S.K., Rasor, M.O'N., Schenker, M.B. Prospective assessment of fecundability of female semiconductor workers. *American Journal of Industrial Medicine*, **28**:817-831, 1995.

62. Eskenazi, B., Gold, E.B., Lasley, B.L., Samuels, S., Hammond, S.K., Wight, S., Rasor, M.O'N., Hines, C.J., Schenker, M.B. Prospective monitoring of early fetal loss and clinical spontaneous abortion among female semiconductor workers. *American Journal of Industrial Medicine*, **28**:833-846, 1995.
63. McCurdy, S.A., Pocekay, D.E., Hammond, S. K., Woskie, S., Samuels, S. Schenker, M.B. A Cross-Sectional survey of respiratory and general health outcomes among semiconductor industry workers. *American Journal of Industrial Medicine*, **28**: 847-860, 1995.
64. Pocekay, D., McCurdy, S.A., Samuels, S.J., Hammond, S.K., and Schenker, M.B. A Cross-sectional study of musculoskeletal symptoms and risk factors in semiconductor workers. *American Journal of Industrial Medicine*, **28**: 861-871, 1995.
65. Samuels, S.J., McCurdy, S.A., Pocekay, D., Hammond, S.K., Missell, L., Schenker, M.B. Fertility history of currently employed male semiconductor workers. *American Journal of Industrial Medicine*, **28**:873-882, 1995.
66. Hammond, S.K., Sorensen, G., Youngstrom, R., Ockene, J. Occupational exposure to environmental tobacco smoke. *Journal of the American Medical Association*, 274:956-960, 1995.
67. Maurer, K.F., Cullen, M.R., Garcia, M.E., Erdil, M., Hammond, S.K. Lead toxicity among bridge workers, 1994. *Morbidity and Mortality Weekly Report.*, 1995.
68. O'Connor, Holford, T.R., Leaderer, B.P., Hammond, S.K., Bracken, M.B. Measurement of exposure to environmental tobacco smoke in pregnant women. *American Journal of Epidemiology*, **142**:1315-1321. 1995.
69. Beavers, J. D., Sweet, C.P., Kenyon, E.M., Hammond, S.K., Smith, T.J., Himmelstein, J.S., Exposure in a household using gasoline-contaminated water: A pilot study. *Journal of Occupational and Environmental Medicine* **38**:35-38, 1996.
70. Milton, D.K., Wypij, D., Kriebel, D., Walters, M.D., Hammond, S.K., and Evans, J.S. Endotoxin exposure-response in fiberglass manufacturing. *American Journal of Industrial Medicine*, **29**:3-13, 1996.
71. Sorensen, G., Stoddard, A., Hammond, S.K., Hebert, J.R., Avrunin, J.S., Ockene, J.K. Double jeopardy: Job and personal risks for craftpersons and laborers. *American Journal of Health Promotion* **10**:355-63, 1996.
72. Hammond, S.K., Hines, C.J., Hallock, M.F., Woskie, S.R., Kenyon, E., and Schenker, M.B. Glycol ether exposures in the semiconductor industry. *Occupational Hygiene* **2**:355-366, 1996.
73. Sorensen, G., et al., for the Working Well Research Group. Working Well: Results from a worksite-based cancer prevention trial. *American Journal of Public Health* **86**:939-947, 1996.
74. Abdollahzadeh, S., Hammond, S.K., and Schenker, M.B. Validity of surrogates for determination of magnetic field exposure for video display terminal users in office settings. *Bioelectromagnetics* **17**:406-410, 1996.
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Charles Perrino
2221 Parker St. Apt G
Berkeley, CA 94704
(510) 845-9975

EDUCATION

University of Florida, Gainesville, MS Environmental Engineering, August 1993
University of California, Berkeley, BA Chemistry, June 1991, ACS certified

EXPERIENCE

UNIVERSITY OF CALIFORNIA, BERKELEY, Berkeley, CA
Lab Manager: Responsible for the management of the School of Public Health Laboratory. The lab focus includes the study of exposure to environmental tobacco smoke and job-related public health hazards, and characterizing pollutants in the Caldecott tunnel. Responsibilities include: operate Hewlett Packard GC, GC/MS and HPLC instruments with NT Workstations; supervise the daily activity of post-docs, doctoral students and undergraduate students; develop analytical methods; maintain GLP and safe lab practice; meet environmental requirements; order equipment; and prepare analytical data for clients. Dates: April 1996-present.

SANDIA NATIONAL LABORATORIES, Livermore, CA
Scientist: Responsible for the management of the analytical chemistry lab on-site which supports many research interests with the use of ICP/MS, GC/MS, and Ion Chromatography. Duties included project management, operation and maintenance of analytical instruments, data interpretation, and report presentation. Analytical support provided to teams researching power electric vehicles, non-proliferation of nuclear materials, and weapons destruction by environmentally sensitive techniques. Obtaining a high-level security clearance was required for this position. Dates: 9/94-Present.

U.S. DEPARTMENT OF AGRICULTURE, WESTERN REGION RESEARCH CENTER, Albany, CA. Organic Chemist: A member of the food chemistry group; projects involved the chemical analysis of food processing by-products and the evaluation of their toxicity. Methods included headspace analyses, distillation, chromatography (HPLC, GC/MS, GC/IR), and organic synthesis.
Dates: 12/93-9/94.

LAWRENCE BERKELEY LABORATORY, Berkeley, CA
Research Assistant: Collected thermodynamic data of organic acids pertinent to the study of ground water leaching at nuclear waste repository sites. Used computer-aided auto-titrators for analyses. Prepared and characterized Organo-metallic complexes and oxides of potassium, cobalt, and nickel.
Dates: 5/92-8/92, 6/91-12/91.

BAY AREA ENVIRONMENTAL, INC., Richmond, CA
Lab Assistant/Hazardous Materials Technician: Responsible for successful EPA certification of methods for determining trace metal content in hazardous waste. Techniques included wet-chemical digestion of samples and Flame Atomic Absorption Spectroscopy. Performed site inspections of potentially contaminated sites and prepared risk assessment reports. Dates: 4/90-4/91

PUBLICATIONS

Synthesis and Occurrence of Oxoaldehydes in Used Frying Oils, Journal of Agriculture and Food Chemistry, V.43, p.22-26, 1995, with Dr. Gary Takeoka

Magnetic characterization of calcium-nickel-potassium oxide catalysts, Journal of Materials Research, V.9, No. 1, Nov. 1994, with Dr. Dale Perry

Temperature and Ionic Strength Dependence of Nitrilotriacetic Acid Protonation Constants, Journal of Solution Chemistry, V.5, No.5, June 1991, with Dr. Heino Nitsche

REFERENCES

Dr. Katharine Hammond, Professor, School of Public Health, UC Berkeley, Berkeley, CA
Please do not contact without permission.

Dr. Michael Annable, Professor, Dept. of Environmental Engineering, University of Florida, Gainesville, FL. Phone: (904) 392-0841.

Dr. Dale Perry, Research Scientist, Lawrence Berkeley Laboratory, Berkeley, CA.
Phone: (510) 486-4819.

Dr. Leroy Chauffe, Chair and Professor of Chemistry, California State University, Hayward, Hayward, CA.
Phone: (510) 885-3452.

INTERESTS:

Hockey, Basketball, Fishing, Bass Guitar, Golf, Computers, Music

BIOGRAPHICAL SKETCH

Janet M. Macher **Co-Investigator**

Ottawa University, Ottawa, Kansas	B.A.	1972	Biology
University of California, Berkeley, California	M.P.H.	1978	Industrial Hygiene & Microbiology
Harvard University, Boston, Massachusetts	Sc.D.	1984	Industrial Hygiene & Microbiology

Professional Experience

1977-1978 Environmental Health and Safety Assistant, U.S. Public Health Service Hospital, San Francisco, Calif. Evaluated the microbiological quality of the water supply to the hospital's dialysis unit, and assisted on inspections for infection-control, fire, earthquake, electrical, and physical safety.

1978-1979 Research Assistant, Naval Biological Laboratory, University of California at Berkeley, Calif. Studied accidental release of genetically altered microorganisms during routine laboratory procedures.

1979-1984 Biological Safety Officer/Teaching Fellow, Department of Environmental Science & Physiology, Harvard University, Boston, Mass. Assisted in a graduate course on environmental health sciences and developed training material and taught a National Institutes of Health-sponsored course on certification of biological safety cabinets.

1984-1985 Guest Researcher, Environmental Microbiology Institute, National Defense Research Establishment, Umeå, Sweden. Evaluated the efficiency of samplers for bioaerosol collection and continued dissertation research evaluating workplace bioaerosol hazards.

1985-present Air Pollution Research Specialist, Environmental Health Laboratory, California Department of Health Services, Berkeley, Calif. Develop and evaluate methods to identify, measure, and control bioaerosols in residences and non-industrial workplaces. Evaluate the performance of air sampling instruments for collecting and identifying bacteria and fungi.

Publications

Macher, JM. Optimization of a procedure to isolate culturable microorganisms from carpet dust. *Indoor Air* (submitted).

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Miller, SL, JM Macher. Evaluation of a methodology for quantifying the effect of room air ultraviolet germicidal irradiation on airborne bacteria. *Aerosol Sci. Technol.* (in press).

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RESUME
FREDERICK W. LURMANN
President

Sonoma Technology, Inc.

1360 Redwood Way, Suite C
Petaluma, CA 94954-1169
707 / 665-9900
Fax 707 / 665-9800
www.sonomatech.com

Educational Background

B.S. Mechanical Engineering, University of California, Santa Barbara, 1971
M.S. Mechanical and Environmental Engineering, University of California, Santa Barbara, 1975

Professional Experience

Mr. Fred Lurmann, in addition to his responsibilities as president of Sonoma Technology, Inc., manages photochemical and aerosol modeling studies, human exposure assessment studies, and aerometric data analysis studies. He was principal architect of the UAM-AERO model that was designed to incorporate size-resolved and chemically-resolved aerosols into the Urban Airshed Model (UAM) for the California Air Resource Board (ARB) and the South Coast Air Quality Management District (SCAQMD). He has developed semi-empirical methods (regression models) for simulating the atmospheric chemistry leading to sulfate, nitrate, and organic PM for numerous simple air quality models, including the CALPUFF model and UAM-LC model. He has applied and evaluated aerosol models for episodic and annual average applications. He was the team leader for the ARB-sponsored project for developing a PM_{10} modeling framework for the San Joaquin Valley. Currently, he is involved (with Caltech and others) in the development of a computationally efficient acid deposition model for California (SAQM-AERO). In addition, he has completed independent statistical model performance evaluations of ozone modeling submitted in support of 24 ozone SIPs (for the EPA) and of UAM-V model applications for the eastern United States for the Ozone Transport Assessment Group (OTAG).

Mr. Lurmann is directing the exposure assessment element of ARB's ten year "Epidemiologic Investigation to Identify Chronic Health Effects of Ambient Air Pollutants" study (with the University of Southern California), which involves indoor, outdoor, and personal measurements of pollutant exposures, time-activity surveys, and development of exposure models for ozone, NO_2, and PM_{10}. He is involved with Kaiser Permanente and UC Berkeley in epidemiologic studies of associations between air pollution and daily mortality and morbidity in California, which are sponsored by the EPA and SCAQMD.

Prior to joining STI, Mr. Lurmann was a private consultant for three years and a senior scientist/program manager with Environmental Research & Technology, Inc. (ERT) for ten years. He has been a leader in the development and evaluation of a number of Eulerian and Lagrangian air quality models, including the ELSTAR, PLMSTAR, MESOPUFF, ADOM, and alternate versions of the UAM model. He has directed numerous studies on the development of atmospheric chemical mechanisms for $VOC/NO_x/SO_2$ mixtures (e.g., the ERT/SAPRC mechanism), including programs for anthropogenic and biogenic VOCs, and programs to evaluate mechanisms using environmental chamber data. He has condensed and implemented chemical mechanisms for use in grid and trajectory models for many years.

Mr. Lurmann has extensive experience applying photochemical models and PM_{10} models for development of control strategies. He has directed studies to evaluate alternate control strategies for ozone attainment, to intercompare models such as the UAM/CB4 and UAM/SAPRC, to apply models to aid in the design of field experiments, and to assess compliance of new and modified facilities with air quality regulations. Furthermore, he has been an invited participant in over 20 air quality modeling conferences and workshops.

Mr. Lurmann's current research interests mostly involve atmospheric ozone and PM_{10} modeling and data analysis. He is currently a member of the Scientific, Technical, and Modeling Peer Advisory Group for the South Coast Air Quality Management District and a former member of the ARB Modeling Advisory Committee.

Memberships

Air & Waste Management Association
International Society for Exposure Analysis
American Association for Aerosol Research

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RESUME
PAUL T. ROBERTS
Executive Vice President

Sonoma Technology, Inc.

1360 Redwood Way, Suite C
Petaluma, CA 94954-1169
707 / 665-9900
Fax 707 / 665-9800
www.sonomatech.com

Educational Background

B.A. Chemical Engineering, Rice University, 1969
M.Ch.E. Chemical Engineering, Rice University, 1970
Ph.D. Environmental Engineering Science, California Institute of Technology, 1975

Professional Experience

Dr. Roberts joined STI in 1986. At STI, he has designed and managed a number of air quality field, data management, and data analysis projects. Most of these projects have involved the use of field data and analysis methods to understand important meteorological and air quality phenomena; to develop, apply, and evaluate meteorological and photochemical models; and to evaluate the effectiveness of ambient air quality and meteorological networks in meeting various regulatory requirements. He had a major responsibility for program management and participant coordination for the Southern California Air Quality Study (SCAQS). He designed and managed a major ozone field study and the resulting data management and data analysis activities for the Sacramento area, and designed and managed the field measurements and data analyses for the MMS-sponsored Gulf of Mexico Air Quality Study, the EPA-sponsored Paso del Norte Ozone Study, and a long-term epidemiologic study in Southern California. He is the Observations Coordinator for the 1995-1997 NARSTO-Northeast Air Quality Studies and helped to plan the data management, observation, QA, and data analysis activities.

Dr. Roberts designed and managed the preliminary data analysis activities and the analysis of boundary condition field data for the San Joaquin Valley Air Quality Study. He has managed two major projects to quantify the contribution of transported pollutants to downwind ozone violations in California air basins. He also led analyses of meteorological and air quality data for the SCAQS, for the Lake Michigan Ozone Study, and for NARSTO-Northeast. Dr. Roberts has led the development and presentation of a 3-day PAMS data analysis workshop for EPA.

Dr. Roberts was also the Measurement Coordinator for the multiyear SCENES Visibility Study in the southwest U.S. He managed STI's activities in the ARB PM_{10} methods assessment project; the Sacramento, San Diego, Lower Lake Michigan, Gulf Coast, and San Luis Obispo Ozone Scoping Studies; and the RESOLVE Study data management and case study analysis.

From 1981 to 1986, Dr. Roberts was Chairman of the API Visibility Task Force, led a WOGA group which sponsored work on PM_{10} in California, was a consultant to the environmental affairs group of Chevron, and testified at Federal hearings. From 1975 to 1986, he planned and directed research and development projects at Chevron Research Company and helped apply the results to operating plants in various Chevron refineries. Project areas included refinery emissions and wastes, lube oil processing, delayed coking, hydrocracking, and processing of coal, tar sands, and shale oil. He was also involved in numerous methods development and methods evaluation projects.

In graduate school, Dr. Roberts developed the flash vaporization technique for measuring nanogram levels of particulate sulfur and carried out research on the transformation of SO_2 to particulate sulfur in Los Angeles. He also participated in the ARB ACHEX and the EPA RAPS.

Dr. Roberts was a member of the California Inspection and Maintenance Review Committee during 1994-1995, has served as an EPA Peer Reviewer since 1995, and is an expert on Victorian architecture in the San Francisco Bay Area.

Memberships

Air & Waste Management Association

Journal Articles

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Patents

Patents issued and applied for in coal gasification, pollution control, and high pressure reactor design.

RESUME
ANN ELIZABETH (BETH) WITTIG
Senior Air Quality Analyst

Sonoma Technology, Inc.

1360 Redwood Way, Suite C
Petaluma, CA 94954-1169
707 / 665-9900
Fax 707 / 665-9800
www.sonomatech.com

Educational Background
B.S. Chemical Engineering, University of California at Los Angeles, 1993
Ph.D. Chemical Engineering, University of Texas at Austin, 1998

Professional Experience

Dr. Wittig joined STI in January 1999. At STI, her primary responsibilities include the design and coordination of air quality field studies and the validation, quality control and analysis of air quality data.

Dr. Wittig is the Field Operations Coordinator for the $2.6MM STI Anchor-Site program for the California Regional $PM_{10}/PM_{2.5}$ Air Quality Study (CRPAQS) in the central San Joaquin Valley of California. The annual field measurement portion of CRPAQS will begin in November 1999 and continue into early 2001; Dr. Wittig will be responsible for more than 40 field instruments and systems at four sites and 1 tower. The winter intensive field portion of CRPAQS will occur in December 2000 and January 2001; Dr. Wittig will be responsible for 115 additional instruments and systems at 11 sites and 2 towers. She has designed all of the STI measurement activities and she has coordinated the preparations for field installation and operations, including instrument specifications and ordering, site logistics, sampling and calibration strategies, instrument checkout and installation, instrument trouble shooting, and operator training and supervision. For the CRPAQS, Dr. Wittig is relocating to Bakersfield to oversee the measurement activities.

In graduate school, her doctoral research focused on both the theoretical and experimental analysis of ambient hydrocarbons. Dr. Wittig performed a detailed sensitivity analysis of CMB 8.0 and identified numerous technical issues with the program. She used source emission and ambient data in and around Houston (Harris County, Texas) to investigate the response of the model to issues such as the availability of accurate source profiles, the use of collinear source profiles, and the availability of unique tracer species. This sensitivity analysis led her to the development and investigation of a new chemical mass balance tool to identify hydrocarbon sources and evaluate ambient hydrocarbon emission inventories. She also investigated the potential of particular sources of hydrocarbons to form ozone and particulate matter in the Central Texas region.

While a graduate student, Dr. Wittig was completely responsible for the establishment and maintenance of the University of Texas at Austin air quality research labs. She was responsible for the operation and maintenance of the hydrocarbon analysis equipment as well as the construction of related apparatus. She developed protocols for the analysis, cleaning, preparation and logging of the hydrocarbon sampling equipment and samples and oversaw the work of several undergraduate and graduate student researchers. She also processed and determined the chemical composition of approximately 275 hydrocarbon samples that were collected during an intensive Central Texas air quality study. As an undergraduate student, Dr. Wittig used FTIR microscopic spectroscopy to identify the chemical composition of organic aerosols and performed volatilization analyses of inorganic aerosols collected in a low-pressure cascade impactor. She also aided in the design of an indoor smog chamber.

As a result of her coursework, Dr. Wittig has developed a strong background in pollution control technologies, multimedia environmental assessment, and air pollution chemistry. In addition, she has had considerable teaching experience.

Dr. Wittig is skilled in the FORTRAN, OCTAVE, HTML, and UNIX programming languages. She is experienced with most machining processes and knows the maintenance and operation of GC-MS, GC-FID, FTIR, and hydrocarbon pre-concentrator instruments, and ambient air monitoring equipment (PM, VOC, NO_X, O_3).

Memberships and Awards
Air & Waste Management Association
Sigma Xi
Received the A.I.Ch.E. award for teaching assistant excellence at UCLA

Publications and Presentations

Wittig A.E., Main H.H., Roberts P.T., and Hurwitt S.B. (1999) Analysis of PAMS data in California Volume III: Trends analysis of California PAMS and long-term trend air quality data (1987-1997). Report prepared for the U.S. Environmental Protection Agency, Research Triangle Park, NC, STI-998393-1885-FR, May.

A. Wittig and D. T. Allen, "Hydrocarbon source allocation using Chemical Mass Balances: characterizing uncertainties for Houston," to be submitted to *Atmospheric Environment*, 1999.

A. Wittig and D. T. Allen, "Ozone formation potentials for urban regions influenced by aged hydrocarbon emissions," to be submitted to *Atmospheric Environment*, 1999.

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A. Wittig, "Atmospheric hydrocarbon chemistry in Central Texas," Ph.D. Thesis, University of Texas at Austin, 1998.

A. Wittig, "Uncertainties in hydrocarbon source allocations determined using the Chemical Mass Balance tool: a case study using Houston data," International Symposium on Measurement of Toxic and Related Air Pollutants, PAMS Session, September 1-3, 1998.

A. Wittig and D. T. Allen, "Source allocation of reactive hydrocarbons at the Houston Galleria site," progress and final reports to the Texas Natural Resource Conservation Commission, July 1997-May 1998.

A. Wittig and C. Wiedinmyer, "Ambient sampling in the Austin area: summer 1996," ESP Spring Research Review Meeting, April 10-11, 1997.

Steven D. Colome, Sc.D.
Adjunct Professor
University of California, Los Angeles
School of Public Health

Professional Positions:

1998-present	Adjunct Professor, UCLA School of Public Health
1997-1998	Visiting Professor, UCLA School of Public Health
1988-present	Principal, Integrated Environmental Sciences
1981-1988	Assistant Professor, School of Social Ecology, UC Irvine
1977-1980	Affiliate, Energy and Environmental Policy Center, Harvard University
1975	Statistician, the Massachusetts Division of Air and Hazardous Materials
1973-1974	Evaluator, Health Center, Columbia Point, Mass.
1973	Research Fellow, Harvard Medical School
1972	Research Assistant, Stanford University Medical School

Education:

1981	Sc.D., Environmental Health Sciences, Harvard University
1972	S.B., Biology, Stanford University

Honors and Awards:

Bird Corporation, R.W. Johnson Foundation and U.S. EPA Fellowships. Nominee, Society of Fellows, Harvard University. Sealco Corporation, Helms Foundation and California Scholastic Federation Scholarships.

Professional Committees/Advisory Boards:

Chair, External Peer Review Committee, EPA CO Criteria Document 1998
Treasurer, International Society of Exposure Analysis (1994-1996)
Consultant to EPA Science Advisory Board CASAC (1992-present)
Chair, Health Effects and Exposure Committee, AWMA (1989-1991); American Lung Association, Indoor Air Pollution Technical Advisory Committee (1988-present); American Lung Association, National Air Conservation Commission (1986-1988); Advisory Council to SCAQMD Board (1988-1996); Technical Advisory Council, SCAQMD (1981-1988); Chair, Health Effects Committee, SCAQMD (1982-1985); Advisory Board, UC Irvine, Hazardous and Toxic Substances Program (1989-present).

Reviewer:

Environmental Science and Technology, Atmospheric Environment, International Journal of Exposure Analysis (Assoc. Editor 1991-1994), Journal of the Air and Waste Management Association (Assoc. Editor, 1995-present), Indoor Air

Research Interest:

Human exposure assessment, environmental epidemiology, indoor air quality, regional exposure modeling, health effects assessment.

Selected Publications in Environmental Science:

Colome, S.D., Wilson, A.L., Tian, Y., Becker, E.W., Behrens, D.W., Billick, I.H. Garrison, C.A., 1998: Carbon Monoxide Concentration Distributions Inside and Outside of Residences in California. Submitted for publication.

Avol, E.L., Navadi, W.C., Colome, S.D., 1998. Modeling Ozone Levels in and around Southern California Homes. *Environmental Science and Technology*, 32(4): 463-468.

Cortes, M., Romieu, I., Colome, S., Mercado, A., Ruiz, S., Palazuelos, E., Hernandez, M., 1997. Evaluation of Ozone Levels in Different Microenvironments Using Passive Ozone Monitors and Predictors of Indoor-Outdoor Ratio in Mexico City. In press: *Journal of the Air and Waste Management Association.*

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Abbey, D.E., Colome, S.D., Mills, P.K., Burchette, R. Lawrence Beeson, W., Tian, Y.. 1993: Chronic Disease Associated with Long-Term Concentrations of Nitrogen Dioxide. *Journal of Exposure Analysis and Environmental Epidemiology*, 3(2):181-202.

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Hall, J.V., Winer, A.M., Kleinman, M.T., Lurmann, F.W., Brajer, V., Colome, S.D., 1992. Valuing the Health Benefits of Clean Air. *Science*, 255:812-817.

Schwab, M., Colome, S. D., Spengler, J. D., Ryan, P. B., and Billick I. H. 1990: Activity patterns applied to pollutant exposure assessment: data from a personal monitoring study in Los Angeles. *Journal of Toxicology and Industrial Health, Exposure Analysis Section*, 6(6):517-532.

Lambert, W. E., Colome, S. D., and Wojciechowski, S. L.. 1988: Application of end-expired breath sampling to estimate carboxyhemoglobin levels in community air pollution exposure assessments. *Atmos. Environ.*, 22(10):2171-81.

Colome, S. D., Fairshter, R. D., Castaneda, N., Pai, U., Brideshead, T., and Lowe, J.. 1987: Accuracy of Miniwright peak flow meters, abstract supplement. *Am. Rev. Resp. Dis., supplement April 1987, 135*(4), part 2:A118.

Colome, S. D., Wilson, A. L., Becker, E. W., Cunningham, S. J., and Baker, P. E.. 1987: Analysis of factors associated with indoor residential nitrogen dioxide: Multivariate regression results. In: *Indoor Air '87; Proceedings of the Fourth International Conference on Indoor Air Quality and Climate*, B. Seifert, H. Esdorn, M. Fischer, H. Ruden, J. Wegner, eds. Institute for Water, Soil and Air Hygiene, Berlin, FRG. 1:405-409.

APPENDIX B

SAMPLE STANDARD OPERATING PROCEDURES (SOP) AND HOME INSPECTION FORM

- STANDARD OPERATING PROCEDURE FOR THE COLLECTION OF LARGE BURKARD SAMPLES
- OPERATING GUIDELINES FOR THE SMALL BURKARD TRAP USING THE 24 HOUR HEAD ASSEMBLY (DRAFT)
- STANDARD OPERATING PROCEDURE FOR THE COLLECTION OF HOUSE DUST SAMPLES
- DETERMINATION OF THE HOUSE DUST MITE ALLERGENS DER P1 OR DER F1 BY ELISA MONOCLONAL IMMUNOASSAY
- PROTOCOL FOR MEASURING ENVIRONMENTAL ENDOTOXIN USING TRIETHYLAMINE PHOSPHATE BUFFER AND KINETIC LIMULUS ASSAY WITH RESISTANT-PARALLEL-LINE ESTIMATION: THE KLARE METHOD
- PROTOCOL AND QUALITY ASSURANCE PLAN FOR NICOTINE ANALYSIS
- WATERS ION CHROMATOGRAPHY METHOD
- HOME INSPECTION FORM

CHAMACOS
Standard Operating Procedure for the Collection of Large Burkard Samples

SOP #BB-01-04
September 30, 1999

Collection of Large Burkard Samples for the CHAMACOS Study

1.0 PURPOSE

This SOP outlines the procedures for setting up the Burkard Spore Trap as part of the CHAMACOS study. These guidelines explain the preparation of the collection slides as well as the proper method for setting up the sampler and changing the slides. The procedures for handling the slides in the field and transporting the slides to a reader are also described.

2.0 DEFINITIONS

2.1 ADHESIVE - the solution/preparation applied to the trapping surface to which the sample adheres

2.2 CHORI - Children's Hospital Oakland Research Institute, the lab core

2.3 ENVIRONMENTAL SPECIMEN COLLECTOR - the CHAMACOS staff responsible for conducting home visits

2.4 LABORATORY COORDINATOR - the CHAMACOS employee who coordinates the lab core located at CHORI

2.5 LAB MANAGER - the CHAMACOS employee who is responsible for the daily changing of Burkard slides and the handling and transport of all samples

2.6 LARGE BURKARD - another name for the Burkard Spore Trap which collects the sample through the suction of ambient air through the trap and across the collection tape

2.7 READER - a person certified by the National Allergy Bureau of the American Academy of Allergy, Asthma, and Immunology (AAAAI) to identify and count pollen grains and fungal spores

3.0 DISCUSSION

The Large Burkard Spore Trap is an active suction sampler that collects 24-hour or 7-day samples of airborne pollen, spores, and particulate matter in outdoor environments. A 24-hour sample collects particles on a slowly moving slide which is then analyzed by a AAAAI-qualified pollen spore slide reader. A 7-day sample collects particles on a rotating tape which is divided into daily sections and then mounted onto a slide and analyzed by a slide reader. For the CHAMACOS study, 24-hour samples will be collected three times per week.

The Large Burkard will provide global samples of overall counts of pollen and fungal spores. Slides from the same three days of each week will be analyzed by a reader. The data from the reading of Large Burkard samples will be used for descriptive purposes in characterizing seasonal trends relevant to the interpretation of patterns of allergy sensitization and the occurrence of acute respiratory symptoms in infants that are part of the study.

4.0 RESPONSIBILITIES

4.1 The laboratory coordinator is responsible for:
- (a) preparing slides and shipping them to the field office;
- (b) verifying shipments with Zeb Dyer.

4.2 The lab manager is responsible for:
- (a) receiving supplies from CHORI;
- (b) arranging slide changing schedule within daily routine;
- (c) removing/replacing slides three times/week;
- (d) ensuring proper labeling of all slides;
- (e) keeping a log of slide changing/collection;
- (f) sending designated samples to Zeb Dyer;
- (g) communicating with laboratory coordinator regarding supplies and spore trap maintenance issues.

4.3 The environmental specimen collectors are responsible for:
- (a) assisting the lab manager with slide changing if needed;
- (b) assisting the lab manager with spore trap maintenance if needed.

5.0 EQUIPMENT

5.1 Materials

5.1.1 Slide Preparation
- (a) Glass slides
- (b) Kim wipes
- (c) Single-edge razor blade
- (d) Slide storage box - RED - unexposed
- (e) Slide preparation log book
- (f) Artist's paintbrush
- (g) Melenex tape
- (h) Cutting block
- (i) Scotch tape

5.1.2 Field Office Handling
- (a) Slide Storage Boxes - RED - unexposed

SOP# BB-01-04

YELLOW - exposed

- (b) Slide Use log

5.1.3 Slide Changing
- (a) Field carrying case for slide
- (b) Scribe
- (c) Pen
- (d) Tweezers

5.1.4 Shipment
- (a) Slide shipment tube
- (b) Slide storage boxes
- (c) FedEx packing supplies

5.2 Reagents

5.2.1 Slide Preparation

(a) Gelvatol
(b) Lubriseal

6.0 STANDARD OPERATING PROCEDURE

The lab manager will change the Large Burkard collection slide on a daily basis three times per week.

6.1 Preparing slides

Glass slides will be prepared with adhesive in batches. A batch number will be assigned to the slides so that preparation conditions may be referenced if necessary. Slides will be given an "expiration date" after which time they should not be used to avoid problems with adhesive strength. The expiration date will be clearly marked on the slide box.

6.1.1 Clean slide preparation surface with Windex and paper towels.
6.1.2 Lay a piece of Melenex tape on the smooth side of the cutting block and fasten ends of the tape to the cutting block with scotch tape.
6.1.3 Lightly buff the Melenex tape surface with a dry lint-free gauze cloth .
6.1.4 Apply Lubriseal with a soft-bristled artist's brush so that the entire tape is uniformly covered with adhesive.
6.1.5 With fine pointed scissors, carefully cut pieces of Melenex tape 5.5cm long, making sure the cut is as straight as possible.
6.1.6 Choose clean glass micro-slides.
6.1.7 Lay a bead of 10% Gelvatol about the length of the tape along the surface of slide.
6.1.8 Holding the Melenex tape segment with forceps, carefully lower the tape onto the film of Gelvatol on the slide.

6.1.9 Straighten tape segment on glass slide
6.1.10 Make sure that the edges of the slides are clear. This will avoid gumming up the Burkard sampler. The adhesive patch should not be closer than 2 mm from the top and bottom edges of the slide. If cleaning is necessary, grip a single edged razor blade from the dull edge and scrape excess adhesive from the top and bottom of the slide. If additional cleaning is necessary, use an alcohol wipe followed by a Kim wipe to remove excess adhesive.
6.1.11 Lay slide on a heating plate to spread adhesive over tape more smoothly. Let slides dry overnight.
6.1.12 Place slides in storage box.
6.1.13 The individual who prepared the slides will label the storage box, noting batch # and preparation date/time, and the expiration date.
6.1.14 Store in designated location until shipment to field office.
6.1.15 Record slide batch details in log book. For each batch, note the total number of slides prepared, temperature and relative humidity at the time of preparation, and adhesive ingredients.

6.2 Shipping slides to Field Office

6.2.1 Place slides in red slide box.
6.2.2 Label slide box with Batch #.

6.2.3 Wrap slide box in bubble wrap.
6.2.4 Wrap in appropriate FedEx shipping materials.
6.2.5 Notify field office of expected shipment arrival.

6.3 Handling slides at the Field Office/Preparing supplies for slide changing
6.3.1 Don a fresh pair of gloves. Select the slide to be placed in drum.
6.3.2 Label the edge of the slide with date using either a pen or a scribe.
6.3.3 Record slide use in log.
6.3.4 Place slide in slide carrying case to take up to the roof.

6.4 Changing the 24-hour slides
The 24-hour slide should be changed three times per week at approximately the same time each day. To accommodate the reader's Saturday schedule, the changing schedule should be arranged so that all three slides can be sent out on Friday.
6.4.1 Turn the power off.
6.4.2 Lock the revolving head of the sampler by placing the locking pin through the block on the base of the sampler and into a hole in the base of the revolving head.

6.4.3 Swing the latch arm on the top of the sampler to the side to release the head.
6.4.4 Carefully lift the head directly upward and free of the trap and place on a stable surface.
6.4.5 With a finger, push slide in the slide holder down until approximately 1 inch of the slide is free of the holder. Grasp slide using edges and gently pull slide free of the holder.
6.4.6 Insert a newly prepared slide into the slide holder by inserting the slide at the bottom of the glides and sliding up into place. Insert the labeled end first so that the label is at the top of the slide when in the sampler.
6.4.7 Push the entire slide mechanism to its lowest or starting position. Using the supplied key, wind the clock mechanism using gentle counterclockwise turns until just snug. DO NOT WIND TOO TIGHTLY!
6.4.8 Insert 24-hour head back into the trap using glides. Swing latch arm into place and release locking pin at the base of the trap. The trap can tun continuously while changing slides.
6.4.9 Turn power back on and check the air flow with flow meter.
6.4.10 Calibrate air flow intake at 10 psi ± 2%.

6.5 Processing slides at the field office
6.5.1 Place all exposed slides in designated yellow storage box.
6.5.2 Send designated weekly samples to Zeb Dyer.
(a) Ensure that slides are properly labeled.
(b) Secure slide(s) in shipment tube.
(c) Pack shipment tube according to FedEx instructions.
(d) Call Zeb Dyer's office/send email to inform him of shipment arrival.

7.0 RECORDS

7.1 A slide preparation log will be kept at CHORI to record the preparation of all slides. Date/time of preparation, # of slides in batch, temperature/humidity conditions, and adhesive recipe will be recorded.

7.2 The field office will have a slide use/inventory log to accompany each box of unexposed slides it receives from CHORI. As slides are used, their removal from storage will be recorded. This will aid in assuring that slides are used within proper timeframe.

7.3 A weekly slide changing form will be filled out by the lab manager and kept on file at the field office.

7.4 A chain-of-custody form will accompany slides from the field office to Zeb Dyer's office.

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7.5 A log in which the use, maintenance, and problems associated with the large burkard sampler will be kept at the field office.

Operating Guidelines for the Small Burkard Trap Using the 24 Hour Head Assembly (Draft)

Operating Guidelines for the Burkard Trap using the 24 hour head assembly (draft)

C.A. Rogers and M. Muilenberg

1. Preparation of slides

1.1 Lay a piece of Melenex tape on the smooth side of the cutting block. Fasten the ends of the tape to the block with a small piece of scotch tape.

1.2 Lightly wipe the tape clean with a lint-free tissue. If desired, a small amount of solvent can be used (ethanol is recommended). Make sure the solvent has completely evaporated before applying adhesive.

1.3 Apply adhesive (silicone grease or Lubriseal) using a 15 to 20 mm wide, soft bristled, artist's brush. A small amount of adhesive can be worked with the brush and it will go a long way. Make sure the entire tape is uniformly covered with a very thin layer of adhesive.

1.4 With fine pointed scissors, carefully cut pieces of Melenex tape 5.5 cm long, making sure the cut is as straight as possible. Be sure to cut off and discard taped ends. Note: to allow some freedom in positioning the tape on the slide, these pieces are longer than the 48 mm required for a daily sample.

1.5 Lay a bead of 10% Gelvatol (a polyvinyl alcohol or "PVA") about the length of the tape, along the surface of a clean microscope slide.

1.6 Holding the Melenex tape segment with forceps, carefully lower the tape onto the film of Gelvatol on the slide. Hint: After touching the end of the tape to the slide, 'curl' the tape downward onto the slide; avoid getting bubbles between the tape and the slide.

1.7 The segment of tape must be positioned properly to cover the area of the slide that will be exposed. There should be roughly ½ cm between one end of the slide and the beginning of the tape. Be sure the tape is positioned perfectly straight on the slide, with even spaces on both sides. The slide should have enough room for labeling at one end.

1.8 Let dry a minimum of 24 hours in a horizontal position before using for sampling. Several slides can be made up at one time and stored in a slide box.

1.9 Before sampling, label the open end of the microscope slide with the date (the day sampling starts), sampling start time and the site name using an ultra-thin permanent ink marker.

Note: Directly applying adhesive to the slide is NOT recommended. Subsequent application of mounting media causes the adhesive to separate from the slide and 'swim' away resulting in a complete loss of sample.

2. Changing slides – <u>Slides should be changed at the same time each day</u>

2.1 Lock the revolving head of the sampler by placing the locking pin through the block on the base of the sampler and into a hole in the base of the revolving head.

2.2 Swing the latch arm on the top of the sampler to the side to release the head.

2.3 Carefully lift the head directly upward and free of the trap and place on a stable surface.

2.4 With a finger, push slide in the slide holder down until approximately ½" of the slide is free of the holder. Grasp slide using edges and gently pull slide free of the holder. Mark the sampling times on the labeled end and place slide in a slide box.

2.5 Insert a newly prepared slide into the slide holder by inserting the slide at the bottom of the glides and sliding up into place. Insert the labeled end first so that the label is at the top of the slide when in the sampler.

2.6 Push the entire slide mechanism to its lowest or starting position. Using the supplied key, wind the clock mechanism using gentle counterclockwise turns until just snug. * Do not wind too tightly!*

2.7 Insert 24 hr head back into the trap using glides. Swing latch arm into place and release locking pin at the base of the trap. The trap can run continuously while changing slides.

3. Mounting slides with Glycerin Jelly

3.1 Using a hot water bath, warm glycerin jelly mixture until melted. On a hot plate or over a flame, gently warm the exposed slide.

3.2 With a pipette or dropper, drop 5 evenly spaced drops of the melted glycerin jelly onto the exposed slide.

3.3 Carefully apply a 22mm X 60mm coverglass onto the glycerin jelly, pressing lightly on the coverglass if necessary to distribute the stain evenly. Reheating the slide may be necessary. Work carefully, excessive manipulation of the coverglass (and jelly) can dislodge and redistribute captured particles.

3.4 Wait at least 30 minutes, or until the jelly has adequately hardened before viewing the slide.

3.5 If desired, the edge of the coverglass can be sealed with clear nail polish to prevent drying of the glycerin jelly.

4. Counting the slide

4.1 First examine the slide for pollen at a magnification of 400X (40X objective with 10x ocular lenses). Afterward, the slide can be examined for fungal spores using oil immersion at 1000X (100X objective and 10X ocular lenses).

4.2 A single longitudinal traverse of the slide should be made roughly 2mm above or below the middle of the slide. Quickly scan the slide to avoid making traverses where bugs or other debris in the orifice have removed or scuffed the trace.

5. Converting counts to concentration

5.1 Measure the field of view of the microscope at both magnifications using a stage micrometer.

5.2 Under normal operating conditions, the flow rate is 10 l/min (or 0.01m^3/min), and the width of the trace is 14 mm. The concentration can be calculated as follows:

$$\text{Conc} = \frac{\#\text{ particles}}{} \times \frac{1}{\text{exposure time (hr)}} \times \frac{\text{trace width (14mm)}}{\text{field of view (mm)}} \times \frac{1}{\text{flowrate } (m^3/\text{min})} \times \frac{1\text{ hr}}{60\text{ min}}$$

$$\text{To simplify this equation: } \frac{\#\text{ particles}}{} \times \frac{1}{\text{exp. time (hr) x field of view (mm)}} \times 23.33$$

For example – with a field of view = 0.445 mm over 24 hours sampled

$$\text{Conc} = \frac{\#\text{particles}}{} \times \frac{1}{24\text{ hr x } 0.445\text{ mm}} \times 23.33 = \text{particles}/m^3 \text{ of air}$$

or: # particles x 2.18 = particles/m^3 of air

Appendix A – Supplies and Materials – Efforts are being made to secure approval to supply premade standardized mountants, however until such time, solutions will need to be made individually. The necessary recipes are given here.

Glycerin Jelly

20 g gelatin
70 ml water
60 ml glycerin (glycerol)
1.2 g Phenol *be very careful with Phenol*

Boil water. Measure 70 ml and add to gelatin. Boil again and mix. Add glycerin and phenol and mix. Add 2-3 drops of **liquid stain** and mix again. Pour into storage bottle and let cool.

10% Gelvatol (polyvinyl alcohol) – for adhering tape to the slide

10 g PVA powder
90 ml water
2 g Phenol *be very careful with Phenol*
Prepare as above.

Liquid Stain

Phenosafranin
Water

To make a saturated stain solution, take the wide end of a toothpick and pick up powdered stain. Carefully place in small tube. Slowly add water a few drops at a time, mixing after each addition. Continue until there are no crystals present in the solution.

What to Buy

Fisher tel. 1-800-766-7000 fax. 1-800-926-1166

Adhesives	Cat. no.	Qty	Price
Dow Corning High Vacuum Grease	14-635-5D	150g	$12.50
Lubriseal Stopcock Grease	14-635M	75g	$14.40
Slides and Coverglasses			
Plain glass microslides	12-550A	144/10Pk	$16.50/$148.00
Fisherbrand coverglasses	12-545J	1oz/10Pk	$11.10/$100.00

Sigma tel. 1-800-325-3010 fax. 1-800-325-5052

Product	Part #	Qty	Price
Phenol (ACS Reagent)	P 4161	25g	$11.60
Phenosafranin	P 5769	250mg	$9.15
Glycerol (glycerin)	G 7757	500ml	$11.50
Gelatin (Type A: Porcine skin) 175 Bloom	G 2625	100g	$8.15

Burkard Manufacturing Co. – FAX +44 1923 774790

Gelvatol (polyvinyl alcohol)	call for price

Contact – for comments or questions regarding this protocol please contact

Mike Muilenberg
Harvard School of Public Health
Dept of Environmental Health
665 Huntington Ave., G33
Boston, MA 02115
(617) 432-0642
(617) 432-3349 fax
mmuil@hsph.harvard.edu

or

Christine Rogers
Harvard School of Public Health
Dept of Environmental Health
600 S. College Ave.
Boston, MA 02115
(617) 432-1860
(617) 432-3349 fax
crogers@hsph.harvard.edu

CHAMACOS
Standard Operating Procedure for the Collection of House Dust Samples

SOP #DS-01-08
October 1, 1999

Collection of Dust Samples for the CHAMACOS Study

1.0 PURPOSE

This SOP outlines the method for collecting dust samples during the home visit component of the CHAMACOS study. These guidelines explain the preparation steps and procedure for collecting indoor household dust samples.

2.0 DEFINITIONS

2.1 ALLERGEN/ENDOTOXIN SAMPLE - a sample collected with the intention of splitting into two separate aliquots, one for analysis of endotoxin levels and the other for the analysis of allergen levels

2.2 CHORI - Children's Hospital Oakland Research Institute, the lab core

2.3 COLLECTION KIT - the supplies compiled for collection of all samples at the home visit

2.4 ENVIRONMENTAL SPECIMEN TECHNICIAN (EST) - the CHAMACOS staff responsible for doing all home visits

2.5 LAB MANAGER - the CHAMACOS employee who is responsible for the handling of all samples at the field office and the transport of samples from Salinas

2.6 LABORATORY COORDINATOR - the CHAMACOS employee who coordinates the laboratory core at CHORI

2.7 PESTICIDE SAMPLE - a sample collected with the intention of future analysis to detect the presence of pesticide residue

3.0 DISCUSSION

Indoor dust samples will be collected by environmental specimen collectors during home visits. These visits will coincide with the participant mother's prenatal care and with the child participant's 6 and 12-month well-baby visits. The house dust samples will eventually be analyzed to identify and quantify endotoxin content in the household as well as determine the presence of common allergens. Additional samples will be collected and banked that may be eventually analyzed for pesticide residues.

All dust samples designated for allergen and endotoxin analysis will be collected using the HVS3 vacuum with a MediVac pre-filter attachment. Samples designated for pesticide residue analysis will be collected using the normal set-up of the HVS3 vacuum cleaner with the possibility of using the HVS3 furniture attachment.

Two-three dust samples for allergen/endotoxin analysis and one sample for pesticide analysis will be collected at each home visit. A sample of the participant's bed, a sample from the kitchen (kitchen/living room if the area is

combined), and a sample from the living room area (if it is separate) will be collected with the MediVac-modified unit. A larger sample from a central living

area will be collected for pesticide analysis. Each of the samples collected with the MediVac-modified unit will be split into separate aliquots for endotoxin and allergen analysis in the CHORI lab.

Sieving of the allergen/endotoxin samples will be done during collection by utilizing the internal filter screen on the MediVac adapter. The pesticide sample will remain unsieved until the time of analysis. In the field, all samples will be kept on blue ice with dessicant to ensure that degradation does not take place. Environmental specimen thechnicians will transport all samples to the field office where they will remain until weekly deliveries to CHORI are possible. At the field office, all allergen/endotoxin dust samples will be kept refrigerated and all pesticide samples will be frozen. At CHORI, allergen and endotoxin aliquots will be separated and allergen samples will be extracted. The extracted allergen sample and all endotoxin and pesticide samples will be stored at -70°C until they are analyzed.

4.0 RESPONSIBILITIES

4.1 The environmental specimen technicians are responsible for:

(a) arranging the home visit schedule;
(b) assisting in the compilation of collection kits;
(c) discussing procedure/purpose with family members during home visit;
(d) performing collections following protocols;
(e) following proper field handling instructions for all samples;
(f) delivering collected samples to field office;
(g) maintenance of vacuums and filters;
(h) inventory and stock of collection materials and equipment.

4.2 The lab manager is responsible for:

(a) compiling collection kits;
(b) receiving all collected samples;
(c) entering all relevant collection data into database;
(d) refrigerating/freezing all samples and ensuring proper dessication;
(e) shipping all samples to CHORI on a weekly basis.

4.3 The laboratory coordinator is responsible for:

(a) receiving all shipments from the field office;
(b) dividing all samples into proper aliquots;
(c) extracting all allergen sub-samples;
(d) storing all endotoxin, pesticide, and extracted allergen samples;
(e) entering all processing data into the database;
(f) sending selected samples to reference laboratories.

5.0 EQUIPMENT

5.1 Collection Supplies

5.1.1 Vacuum supplies

(a) HVS3 vacuum cleaner

7 HVS3 furniture attachments

(c) 250 mL FEP Nalgene collection bottle

(d) (3) clean, dry MediVac collection heads

7 Paper clips

8 Log book

9 Collection Forms (Sample collection & Chain-of-custody)

10 Plastic bags for used MediVac collection heads

11 Plastic bags for used FEP bottles

5.1.2 Home Visit Collection Kit

The collection kit will be in a large labeled (by HSN#) plastic bag containing the following:

(a) (3) Nylon collection filters

(b) (3) small labeled (barcodes) plastic bags

(c) Paper clips

7 (3) Glaccine envelopes

8 (3) Dessicant packs

9 (1) small labeled (barcode) glass jar

10 (1) small plastic bag (for glass jar)

The kit will be assembled so that each nylon filter is within a glaccine envelope inside of a labeled plastic bag with a dessicant pack.

5.1.3 Other Collection Supplies

(a) Small cooler

(b) "Blue" ice

(c) Latex gloves

(d) Timer/stop watch

(e) Measure meter

(f) Masking tape

7.1 Cleaning Supplies

5.2.1 Squirt bottle (s)

5.2.2 Reagent grade Isopropanol

5.2.3 Deionized water

5.2.4 Laboratory glassware detergent

5.2.5 HDPE cleaning tray

5.2.6 Latex gloves

5.2.7 Sheet of clean plastic (garbage bag)/Plastic tray

5.2.8 Lab brushes

5.2.9 Toothbrush

5.2.10 Tweezers

5.2.11 Wide mouth glass jar or stainless steel bucket

5.2.12 Clean zip lock bags

5.2.13 New, clean, large plastic garbage bags

7.2 Shipping Supplies

5.3.1 Styrofoam shippers

7.1.1 "Blue" Ice

7.1.2 Dry Ice

7.1.3 Bubble wrap

7.1.4 Packing supplies

6.0 STANDARD OPERATING PROCEDURE

6.1 Preparation

6.1.1 Collection kit preparation at the Field Office.

(a) Lab manager compiles collection kits for confirmed home visits. ESTs compile all other vacuum and collection supplies.

(b) Make sure each nylon filter is in a labeled plastic bag and that all three collection heads are clean.

7 Make sure that a clean FEP Nalgene collection jar for the HVS3 is included and that the proper transfer vial is in the collection kit.

(d) Verify directions, phone number, and contact person of scheduled home visit.

6.1.2 Preparation for collection at the Home

(a) Communicate with household participants regarding the nature of the collection procedures and the types of things you will be doing. This should include informing them that you will first be walking through the house to determine the order/type of collection and that you will ultimately be removing their bedding. It should also be explained to household members that it is preferable to have as little human activity as possible in the designated areas during collection.

(b) Along with the EST who will be doing the walk-through questionnaire part of the visit, do a brief assessment of the size and make-up of house in order to determine the sample collection sites. The participant should be asked questions regarding where the study subject sleeps (if the subject sleeps in multiple locations, the place where they spend 3-4 nights/week should be designated) and spends the most time in the house. Based on the answers to these questions and the type of floor surfaces available, the three-four collection sites should be chosen.

(c) Outline the collection plan on the third sheet of the sample collection form. This should include doing a rough sketch of the floor plan of the house and labeling the spots where samples are collected. Communicate your plan to the other member of the EST team so that the walk-through questionnaire does not disrupt sample collection. **The Mini-Burkard samples (see MB SOP) should be collected <u>BEFORE</u> the vacuum samples are collected.**

Vaccum outline should proceed as follows:

(1) Choose a site in the central living area to collect the pesticide sample. This may involve sampling furniture and/or various floor surfaces. *(1 sample)*

(2) Sample from participant's bed. (*1 sample*)

(3) Sample from the kitchen area. (*1 sample*)

7 If the kitchen and living room area are combined or are the same, collect a third allergen/endotoxin sample only if the surface of the "living room area" is different from the

"kitchen area." If the living room area is separate from the kitchen, collect a 3rd allergen/endotoxin sample within this area and note floor surface. *(1 sample)*

(d) Inform the participant of your plan. i.e. "First I will be vacuuming your bed for a few moments. Afterwards I will be vacuuming the kitchen and living room floors." Ask for permission to remove/rearrange bedding. Besides making the participant more aware of the procedure, this will hopefully help keep people out of these areas until after the collection is finished.

6.1.3 Setting up the HVS3 for pesticide sample collections

(a) Don a pair of latex gloves.

(b) Remove cleaned FEP Nalgene bottle from plastic bag and screw into collection port on the vacuum.

(c) Attach clean collection head or furniture attachment onto vacuum unit.

(d) Attach additional tubing if necessary.

(e) Ensure that all seals are tight.

(f) Plug in vacuum.

6.1.4 Setting up the MediVac-modified vacuum for allergen/endotoxin sample collections

(a) Attach MediVac tubing and head to HVS3 unit.

(b) Don a pair of latex gloves.

(c) Remove the nylon filter from plastic bag and sandwich between vacuum hose and a clean collection head (see **DIAGRAM 1**).

(d) Ensure that collection head is screwed into vacuum hose so that a seal is formed.

(e) Plug in the vacuum.

6.2 Collection of pesticide sample

6.2.1 Choose a square meter in the central living area. Measure with ruler or meter and mark off with masking tape.

7.1.1 Vacuum the square meter in such a manner that four double passes of the entire surface are performed.

7.1.2 Record sampling time.

7.1.3 Carefully unscrew FEP Nalgene collection bottle.

6.2.5 Using anti-static brush, transfer dust sample from Nalgene bottle to labeled glass jar. Put jar inside of plastic bag and back into collection kit.

6.3 Collection of allergen/endotoxin samples

A new collection head must be used for each separate location's sample.

6.3.1 Collecting the Bed Sample

(a) Using a measuring tape or meter, mark off the section of the bed to be used. Choose an area at the head of the bed if possible. Record area on collection form.

(b) Vacuum the upper surface of the mattress or mattress pad. If possible, continue by vacuuming the pillow, bottom sheet, blanket(s), and quilts. Total vacuuming time in the bed should be at least 2 minutes. Ensure that you have done four double passes of the surface.

(c) If there is a mattress pad, you do not need to sample beneath it.

(d) Mattresses are to be sampled even if they are encased in plastic or another dust-mite impermeable cover. These types of encasements should not be removed.

(e) Use timer to ensure that sampling time is properly recorded. 2 minutes is the minimum suggested time for sampling. Do not vacuum for less than this time but do continue on for longer if sufficient sample is not collected. Record sampling time.

(f) Before turning the vacuum unit off, turn the MediVac attachment upright. Agitate any material blocking the filter so that any loose dust on the pre-filter is collected onto the nylon filter. Turn the vacuum off.

(g) Slowly unscrew collection head being careful not to lose any dust from the nylon collection filter. Fold the nylon filter so that the collected dust is concentrated at the center of the square of material. Fold the outer edges over the sample so that loss is minimized and secure with a paper clip. Place the folded filter in a glaccine envelope. Place the envelope in the properly labeled plastic bag.

(h) Place used collection head in cleaning bag.

(i) Re-make the participants bed with their permission.

(j) Change collection head and filter. See 6.1.4 c and d.

6.3.2 Collecting the Kitchen or Kitchen/Living Area Sample

(a) Using a measuring tape or meter, measure the area of the floor you will be sampling. This may be an approximate value. Record area on collection form.

(b) Turn vacuum on and collect sample for at least 2 minutes. Modify collection time based on the amount of sample you are able to retrieve.

(c) Collect sample from central kitchen, along baseboards, near appliances, and in any other areas that are open. Do not sample from behind the refrigerator or other areas that are inaccessible.

(d) Follow steps 6.3.1 (e) - (h) to put sample away properly.

(e) If the kitchen is combined with the living area and the kitchen floor surface you have sampled is NOT the same as the floor surface in the part of the area considered to be the living area OR if the living room

separate, move on to 6.3.3 to collect a third sample. Change collection head and filter, following steps 6.1.4 (c) & (d).

6.3.3 Collecting a sample from a separate living room or a combined living room with a different type of floor surface.

(a) Mark off a square meter with ruler or meter.

(b) Vacuum square meter for at least 2 minutes. Record elapsed time on collection form. Make sure to do four double sweeps of the surface.

(c) Follow instructions 6.3.1 (e) - (h).

(d) Place used collection head in cleaning bag.

7.2 Handling and Transport of samples in the field

6.4.1 Ensure that all bagged samples are labeled properly and contain a dessicant pack.

6.4.2 Fill out all relevant fields of data collection form.

6.4.3 Ensure that pesticide sample has been transferred to the proper labeled jar.

7.1.1 Place all samples in cooler with "blue" ice.

7.1.2 If not returning to the field office, store allergen/endotoxin samples in the refrigerator and the pesticide sample in the freezer.

6.5 Handling at field office

6.5.1 Environmental specimen technicians sign over chain-of-custody form to lab manager.

6.5.2 Lab manager places all bagged samples in refrigerator.

6.5.3 Lab manager will periodically check dessicant (only applicable if sample remains at field office for several days).

6.5.4 Lab manager enters all relevant fields from data sheets into database.

6.5.5 ESCs clean all attachments and collection heads from cleaning bag as well as the HVS3 sampling train.

7 Place the sampler in a well-ventilated cleaning area that is free from dust. The surface should be level and covered with clean plastic.

8 Take care to avoid knocking dirt or dust onto clean plastic. Do not put sampler down except on plastic surface in transport box.

9 With the sample bottle removed, open the butterfly to maximum flow, tip the sampling train back so that the nozzle is 2" off the surface, and switch the HVS3 on.

10 Place a hand covered by a glove over the bottom of the cyclone and alternate closing and opening the cyclone for 10 seconds to free any loose material adhering to the walls of the cyclone and tubing.

11 Remove the cyclone cone, bellows connector, and elbow at the tip of the nozzle tubing from the sampler.

12 Wearing gloves, dismantle gaskets and bellows. Rinse and brush bellows, gaskets, and sample bottle cap with deionized water (DI), detergent, DI again, and isopropanol (IPA), then store in wide-mouth jar or HDPE cleaning tray.

13 Rinse all interior sections of the sampling train with DI, detergent, and then DI. Brush all interior surfaces, rinsing with IPA between brushing. Rinse again with DI and then IPA.
14 Clean brush by rinsing with DI, detergent, DI again, and IPA.
15 Clean catch bottle using same procedures, using the brush to carefully clean threads.
16 Wash wheels with IPA.
17 Dry sampling train with Kim-wipes. Retrieve gaskets and bellows with gloved hand. Assemble. This procedure should take about 30 minutes total.
18 Dry the sampler pieces in air for 20 minutes or by drawing air through the assembled sampler for 5 minutes.
19 Store clean HVS3 in new, large plastic bag.

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6.6 Transport to CHORI

7.1 All endotoxin/allergen samples should be packed in a Styrofoam shipper with "blue ice" packs and bubble wrap.
7.2 Make sure that all bags containing samples are securely closed.
7.3 All pesticide samples should be maintained frozen by packing with dry ice.
7.4 For packing details, refer to SOP#U-01-07

7.0 RECORDS

7.1 Sample collection forms will be filled out for all collections. See Appendix 1.
7.2 ESTs will keep a log book of all maintenance and cleaning performed on the HVS3.
7.3 When samples are transferred to the lab manager, a chain-of-custody form will be filled out.
7.4 The lab manager will create shipping lists and a new chain-of-custody forms when samples are sent to CHORI.

Environmental Health Laboratory Branch
Determination of the House Dust Mite Allergens Der p 1 or Der f 1 by ELISA Monoclonal Immunoassay

Environmental Health Laboratory Branch

Determination of the House Dust Mite Allergens Der p 1 or Der f 1 by ELISA Monoclonal Immunoassay*

Analyte:	Extracted antigens of Dermatophagoides pteronyssinus (Der p 1) and Dermatophagoides farinae (Der f 1)	**Method No:**	
Matrix:	House dust, sieved through a No. 45 mesh screen, 355 um diameter.	**Range:**	0 to 250 ng/ml
Procedure:	Enzyme Linked Immunosorbent Assay (ELISA)	**Precision:**	Der p 1 Dupl. CV = 13.1% Der f 1 Dupl. CV = 17.8%
Date Issued:	May 1, 1997	**Sampler:**	Modified vacuum cleaner (Hoover Sprint 100 or equiv.)
Date Revised:		**Field Blank:**	Vacuum cleaner filter in a sealed plastic bag
		Calibration:	UVa Der p 1 2500 ng/ml UVa Der f 1 2500 ng/ml UVa Reference Standards

1.0 **Summary of Method:**
Samples are collected from specific areas in homes by a modified vacuum cleaner, and the specimens sieved and a weighed amount of dust stored in vials. A saline buffer is added, and any dust mite allergen present (>99%) is extracted. The vials are centrifuged and the liquid supernatant removed and refrigerated until analysis. The foregoing is a summary of the House Dust Sample Method[1,2]. The ELISA method following is concerned with the house dust mite allergens Der p 1 and Der f 1, which are quantitated by means of Microtiter plates coated with allergen specific Monoclonal Antibodies, followed by a second enzyme labeled antibody which reacts with the plate bound allergen. An azino reagent is added, and the resultant colored solution in each Microplate well, whose intensity is proportional to the amount of bound allergen, is read on a Microplate UV Analyzer.

2.0 **Range:**
Overall Range: 0 to 250 ng/ml, equivalent to 0 to 200,000 ng/g, or 0 to 200 ug of allergen per gram of dust.
Optimal Range: 15.6 to 125 ng/ml (liquid standard calibration range).

2.1 **Sensitivity**[3]: 100 ng of allergen per gram of dust.
2.2 **Detection Level:** The lowest point of calibration is 0.5 ng/ml.

* Prepared by W.J. Wehrmeister, Environmental Health Laboratory Branch, 1997.

Laboratory Director Date

3.0 **Precision**[3,4,5]: (within the assay samples of the extraction fluid, n=144)
Der p 1: Duplicate sample CV: 13.1% Triplicate sample CV: 4.8%
Der f 1: Duplicate sample CV: 17.8% Triplicate sample CV: 8.7%

4.0 **Accuracy:**
Two studies[3] (n=52, 132) of Mab ELISA and Mab RIA gave r = 0.90-0.96 for Der p 1 and r = 0.86-0.92 for Der f 1. The sum of Der p 1 and Der f 1 , gave r = 0.85 with Mab RIA AgP_1E3[3]. A comparison[6] of the microscopy dust mite count ratio Der p1/Der f 1 with Mab RIA gave r = 0.89. An interlab comparison study[5] between EHLB and the University of Virginia (UVa) gave r values of 0.98 for both Der p 1 and Der f 1.

5.0 **Interferences**[3]:
There is little (<2%), or no detectable cross-reactivity between Der p 1 and Der f 1 over the range of the control curves. The Dermatophagoides species II does not react. Dermatophagoides microceras may give some cross reactivity, (~5%), but this is not a common species in the U.S.

6.0 **Safety and Laboratory Precautions.**
A Bio-safety Class II Hood, with a HEPA filter and uv lamp, should be used to guard against possible exposure. House dust may harbor bacteria, viruses, human residues, parasites, and insect life such as cockroaches. For personal protection, wear a full lab coat, vinyl gloves, an OSHA particulate air filter, and plastic shoe guards. Working tools should be cleaned in a fume hood with a mild disinfectant such as ethanol. Skin contact with Mabs and other reagents should be avoided. Use sterile techniques to avoid contamination of the Mab and reference solutions. Biomolecular and light sensitive reagents should be quickly capped and refrigerated after use, and shielded from light sources.

7.0 **Apparatus**

7.1 **Safety Equipment**

7.1.1 Class II, Type A/B3 safety hood, with HEPA filter and uv germicidal lamp.

7.1.2 Standard general purpose laboratory fume hood.

7.1.3 Dust/mist respirators, disposable mask type, HEPA.

7.1.4 Gloves; laboratory coat; disposable shoe covers.

7.2 **Analyzers and Associated Equipment**

7.2.1 Microplate UV Analyzer, "UV Max", Molecular Devices[7] of Menlo Park, CA, or equivalent, with software for automated analysis.

7.2.2 Computer, 486 or better, with operating system software.

7.2.3 Microplate washer, manual or automated, Nunc.

7.2.4 Centrifuge, temperature controlled, capable of 2500 rpm, Beckman GPR or equivalent.

7.2.5 Balance, for weighing dust samples and centrifuge tubes.

7.2.6 pH Meter.

7.2.7 Orbital rotator, Scientific Instruments Inc, or equivalent.

7.2.8 Multichannel pipet.

7.2.9 Gilson Autopipets (or equivalents), of ranges 10, 20, 100, 200, 1000, 5000 µl.

7.2.10 Pipet Aide, Drummond, for use with disposable sterile serological pipets.

7.2.11 Immulon II Microwell Plates (Dynatech Co., Alexandria, VA).

7.2.12 Pipet reagent reservoirs for multichannel pipets.

7.2.13 Disposable graduated sterile polypropylene conical tubes, 15 and 50 ml, Falcon.

7.2.14 Microcentrifuge tubes with caps, 1.7 ml.

7.2.15 Vortex mixer, Fisher.

7.2.16 Pipet tips, tube racks, disposable sterile Pasteur pipets.

7.2.17 Glass reagent storage bottles, 500, 1000 ml; volumetric flasks, sterile plastic filtration units; vacuum trap.

8.0 Reagents and Materials

The Reference Standards and Monoclonal Antibodies below may be obtained from the John Hopkins University School of Medicine[8], as a kit.

8.1 Der p 1 Reference Standard UVa 93/03, 2500 ng/ml of allergen.

8.2 Der f 1 Reference Standard UVa 93/02, 2500 ng/ml of allergen.

8.3 Monoclonal Antibody 5H8 Anti Der p 1. Stock solid.

8.4 Monoclonal Antibody 6A8 Anti Der f 1. Stock solid.

8.5 Monoclonal Antibody 4C1-B8-3F8 Anti Der p 1 & Anti Der f 1 (liquid).

8.6 Streptavidin Peroxidase Sigma S 5512 protein, 0.25 mg. (Sigma Co., St. Louis, MO.)

8.7 Albumin, Bovine Sigma A 7030 100 grams.

8.8 Thimerosal Sigma T 8784 1 gram. (preservative)

8.9 ABTS [2'2'-azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid)], Fw 548.7 Sigma A 1888
2 grams.

8.10 Hydrogen Peroxide Sigma H 1009 30%, 100 ml.

8.11 Sodium Azide, NaN_3, FW 65.01. Sigma S 8032 25 gram lot.

8.12 50mM **Carbonate/Bicarbonate Buffer**, pH 9.6

Na_2CO_3	1.59 grams
$NaHCO_3$	2.93 grams
Thimerosal	0.10 grams (preservative)

To make one liter with Millipore filtered deionized water

8.13 (**PBS-T**) Phosphate Buffered Saline, pH 7.4, containing 0.05% Tween 20

NaCl	8.00 grams
KH_2PO_4	0.20 grams
Na_2HPO_4	1.15 grams
KCl	0.20 grams
Thimerosal	0.10 grams
Tween 20	0.50 ml.

To make one liter with Millipore filtered deionized water

8.14 (**1% BSA PBS-T**) 1% Bovine Albumin in PBS-T

Bovine Albumin	1.0 gram
PBS-T Solution	100 ml.

Dissolve over several hours and use a sterile vacuum filtration unit to clarify the solution.

8.15 **Buffer Solution A**

0.1m anhydrous citric acid:	19.21 grams
Millipore filtered deionized water	1.0 liter

8.16 **Buffer Solution B**

0.2m Na_2HPO_4 $7H_2O$ (dibasic)	53.65 grams
Millipore filtered deionized water	1.0 liter

8.17 70mM **Citrate - Phosphate Buffer**, pH 4.2

Buffer solution A	147 ml.
Buffer solution B	103 ml.

To make 500 ml with Millipore filtered deionized water.

8.18 **Stock Antibody Solution** (either allergen)

PBS	1.0 ml.
5H8 (Anti der p 1)	10 mg.
-or-	
6A8 (Anti der f 1)	10 mg.

Resuspend the freeze dried material, then refrigerate and keep from light.

8.19 **Stock Streptavidin-Peroxidase Solution**
Millipore filtered deionized water 1.0 ml.
Strep.-Perox. (Sigma S 5512) 0.25 mg.
Separate into 50 ul aliquots in capped containers and store at -20C.

8.20 **Stock Substrate 1mM ABTS Solution** (coloring agent)
ABTS (Sigma A 1888) 274 mg.
70mM Citrate - Phosphate Buffer 500 ml.
May be stored in an opaque plastic bottle at 4 degrees C. for long time periods (keep from light).

8.21 **Working 2 mM Sodium Azide Solution**
Millipore filtered deionized water 100 ml.
Sodium azide, Sigma S 8032 13 mg.
Store in an opaque bottle and keep refrigerated.

9.0 Prior Field Dust Sampling and Extraction Procedure

For the details of field sampling and dust extraction, see the EHLB Dust Method[1,2], which describes the use of a modified vacuum cleaner to collect dust samples in the field. The vacuum filter and dust sample are dispensed into a plastic storage bag, which is refrigerated. A portion of the dust is sieved, and 100 mg in a vial is extracted with saline buffer. The vial is centrifuged, and the supernatant analyte removed with a Pasteur pipet and refrigerated until the day of analysis. From this point, the method is continued as in the next section, with the preparation of a Mab activated plate.

Der p 1 & Der f 1 Sample Analysis by ELISA Microplate Monoclonal Antibody Immunoassay[9]

Procedure for Microplate Antibody Activation (One Day Prior to Analysis.)

9.1 ☐ **Prepare fresh working Antibody Solution** (5H8-anti Der p 1 -or- 6A8-anti Der f1)
50mM Carbonate-Bicarbonate Buffer 10 ml. in a 15 ml Falcon sterile polypropylene vial.
Stock Antibody Solution (8.18) 10 µl
Dispense immediately as below, with a multichannel pipet and pipet reservoir. Total volumes may be prepared slightly more than 10 ml, if needed, for all of the method working microplate solutions, to insure enough fluid for all of the wells.

9.2 ☐ **Dispense 100 µl of the Working Antibody Solution** to each well of an Immulon Microplate, and incubate the plate overnight at -4 degrees C. (Several plates may be prepared and stored). The wells may be protected at each stage of the analysis, by covering the plate with a fresh plastic film wrap.

Blocking the Plate (Day of Analysis)

9.3 ☐ **Wash the activated plates twice with PBS-T**, (after removing from the refrigerator).

9.4 ☐ **Add 100 µl of 1%BSA-PBS-T** to all wells, and **incubate at room temperature for one hour.**
☐ Time Start: ☐ Time End:

9.5 ☐ **Wash the plate twice** with PBS-T, invert and tap plate, and dry.

Sample Preparation and Addition (During the time period of Blocking, above)

9.6 ☐ **Prepare dilutions of the samples** as below, using the 1.7 ml conical centrifuge tubes with caps.

a) For a 1 to 10 dilution, add 100 µl of analyte to 900 µl of 1%BSA PBS-T to make 1.0 ml.
b) For a 1 to 20 dilution, add 500 µl of sol a) above to 500 µl of 1%BSA PBS-T to make 1.0 ml.
c) For a 1 to 40 dilution, add 500 µl of sol b) above to 500 µl of 1%BSA PBS-T to make 1.0 ml.

9.7 ☐ **Add 100 µl aliquots of the analyte dilutions** (9.6 a,b,c), making a row of 4 samples across the plate. Repeat until the plate is filled, with 21 samples, 2 duplicates, 1 control, and at the top, two rows of standards.

9.8 ☐ **Duplicate QC Samples:** Repeat 2 samples in random locations on the plate.

Working Calibration Standards and Control (to be added to the samples on the above Microplate)

9.9 ☐ **Prepare a Working Calibration Standard** (250 ng/ml) from the UVa Reference Standard.For **Der p 1,** add a 300 µl aliquot of Ref Std 93/03 to 2.7 ml of 1%BSA PBS-T to make 3.0 ml.

-or-

for **Der f 1,** add a 300 µl aliquot of Ref Std 93/02 to 2.7 ml of 1%BSA PBS-T to make 3.0 ml.

9.10 ☐ **Prepare a row of calibration standards on the plate.**
Add 100 µl of the working calibration standard above to the first top well, then doubling dilutionsthereafter (1%BSA-PBS-T), leaving the last two wells in the row as blanks. Repeat for the second row.

9.11 ☐ **Control:** Prepare 125, 62.5, 31.3 ng/ml solutions by adding 100, 50, 25 µl aliquots of Reference Standard into 1900, 1950, and 1975 µl of 1% BSA PBS-T, each to make 2.0 ml. Dispense 100 µl aliquots of each into a random 3 well sample site on the plate.
Samples, Controls and Working Standards may be refrigerated for periods of a week or more.

Incubation of Standards, Samples, and Control on the plate

9.12 ☐ **Incubate for 1 hour**
☐ Time Start: ☐ Time End:

Common Antibody (Biotinylated 4CI)

9.13 ☐ **Prepare fresh Working 4CI Antibody solution.** (Recognizes both Der p & Der f epitopes)
1%BSA PBS-T 10 ml. in a 15 ml Falcon sterile polypropylene vial.
Monoclonal Antibody 4CI-B8-3F8 10 µl

9.14 ☐ **Wash plate 5x** with PBS-T.

9.15 ☐ **Add 100 µl aliquots of the Working 4CI solution** to the plate.

9.16 ☐ **Incubate for one hour.**
☐ Time start: ☐ Time end:

The Avidin Enzyme Conjugate

9.17 ☐ **Prepare fresh Working Streptavidin-Peroxidase Solution.**
1%BSA PBS-T 10 ml in a 15 ml Falcon sterile polypropylene vial.
Stock streptavidin peroxidase solution: 10 µl

9.18 ☐ **Wash Cells 5x** with PBS-T

9.19 ☐ **Add 100 µl aliquots of the Working Streptavidin-Peroxidase.**

9.20 ☐ **Incubate 30 minutes.**
☐ Time start: ☐ Time end:

The Substrate

9.21 ☐ **Prepare fresh Working ABTS Substrate solution** while the streptavidin peroxidase is incubating.
Stock Substrate (8.20) 10 ml.
30% H_2O_2 7 uL.

9.22 ☐ **Wash plate 5x** with PBS-T.

Developing the Plate Color

9.23 ☐ **Add 100 µl aliquots of the Working ABTS Substrate Solution.**
Plate end point color should develop slowly over a period of forty five minutes to an hour. If color occurs swiftly, reduce amount of peroxide on the next analysis.
☐ Time Start: ☐ Time End:

9.24 ☐ **Add 100 µl of 2 mM working Sodium Azide solution,** to retard color development, if needed.

10.0 Calculations

10.1 The **Primary Units** are the label concentrations on the **Reference Standard** from (UVa) John Hopkins, i.e, ng/ml.

10.2 The **Reference Standard** is diluted 1/10 to make a **working calibration standard**, and doubling dilutions made on the Microplate, for 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, 0.977, 0.488 ng/ml standards, and two blanks.

10.3 The proprietary computer program[7] calculates a **four parameter calibration curve** after the results of analysis of the standards above,
$Y = (A-D)/(1 + (X/C)^{B}) + D$, where A, B, C, D are 4 parameter values, Y = Concentration, and X = OD.

10.4 The program next calculates the concentration (ng/ml), of the allergen sample, for each dilution factor and respective optical density (OD), and enters it onto a computer file and table printout.

10.5 The **optimal value** (of the three results for each sample) selected by the analyst, is that closest to the center of the calibration curve, if possible, or is otherwise best representative.

10.6 Calculate the final concentration result, (wt of allergen per gram of dust), as below:

Conc(ng/g) = (opt. value, ng/ml) X (1000mg/g)/(wt of dust sample in mg) X (volume of extraction fluid in ml)

10.7 To convert units from ng/g to ug/g, divide the above result by 1000.

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PROTOCOL FOR MEASURING ENVIRONMENTAL ENDOTOXIN USING TRIETHYLAMINE PHOSPHATE BUFFER AND KINETIC LIMULUS ASSAY WITH RESISTANT-PARALLEL-LINE ESTIMATION:

The KLARE Method

Donald K. Milton, MD, DrPH

Harvard School of Public Health

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ENDOTOXIN SAMPLING

Aerosol Sample Collection:

1. Sampling Cassettes and Media: Use polycarbonate 0.40 μm capillary-pore membranes (Nuclepore) loaded into new, three piece polystyrene cassettes with glass fiber backing pads (Millipore AP40) using sterile technique and endotoxin-free forceps (Figure 1). Backing pads and polycarbonate membranes are endotoxin free as purchased from the manufacturer. However, this must be confirmed for new lots of filters and pads and routinely by use of lab blanks. Close cassettes until needed for sampling with blue plugs inserted into the inlet and red plugs inserted into the outlet or pump side of the cassette. Close cassettes securely using a rubber-padded vice-grip lever and visually inspected to ensure proper seal.
2. Collect samples at 1.5 to 2.5 lpm. Higher flow rates can be achieved with appropriate pumps. Appropriate sampling times depend on the expected endotoxin concentration and particulate loading as well as flow. For levels near background a minimum 1 m^3 sample is required. Cassettes may be used open or closed face depending on working conditions; closed face is often preferred under wet conditions to avoid splashing onto the filters.
3. Pumps with cassettes attached must be calibrated at the beginning and end of sampling due to possible changes in flow from particle loading of capillary-pore membranes. A rotameter or bubble tube (e.g. Gillibrator) can be used in line with the cassette if care is taken to ensure that the devices are clean. Bubble chambers must be cleaned at least monthly and refilled with fresh bubble solution. Laboratory blanks should be tested with the meters before starting a field study by calibration of at least 3 cassettes and comparison of the results with filters placed in the cassettes but not calibrated. There should be no increase in detectable endotoxin level on the calibrated cassettes.
4. Following collection attach a desiccator (Figure 2) to the pump side of the sample cassette and store at 4°C until shipment. Desiccator attachments consist of a tube or sealed cassette filled with dry $CaSO_4$ and a backing pad or glass or cotton wool to prevent desiccant from leaving cassette or tube and entering sampling cassette.
5. Pack samples, with desiccators attached, in plastic bags surrounded by blue ice. Ship samples to the laboratory by over night carrier, ensuring that the samples arrive on a work day.

Background Sample Collection and Field Blanks:

1. For comparison with samples of interest, collect duplicate samples of ambient air out-of-doors and outside of the building envelope or sufficiently removed and up wind of the site of investigation as to be representative of background endotoxin levels.

2. Field blanks (10% or 2 cassettes minimum) should be included in each investigation. Each blank should be calibrated in the same manner as the sample cassettes by the field technician.

Blanks

Blanks are essential to detecting contamination. Sampling indoor environments for low level endotoxin exposures is especially sensitive to contamination and requires frequent analysis of blanks. This is particularly true if a primary standard, such as a Gilibrator that uses soap bubbles, will be used to calibrate flow through the filter cassette.

Definitions:

1. A **lab blank** is a filter placed in a cassette and calibrated, but not weighed.
2. A **field blank** is a filter that is treated exactly the same way as a sample filter (weighed, calibrated, sent to the field, and stored in the same manner), except that it is not used for sampling.
3. A **filter blank** is a filter placed in a cassette but not weighed or calibrated.

Guidelines for Use:

1. **Lab blanks** are used to check for contamination from calibration using the labs Gilibrator. The Gilibrator should be checked for contamination with 2 lab blanks every two weeks. If the Gilibrator is contaminated, it should be thoroughly washed with distilled water, allowed to dry overnight in the hood, refilled with new bubble solution, and tested again. If the lab blanks are still contaminated, the bubble solution should be assayed to see if it is the source of the contamination and replaced as needed.
2. **Field blanks** are used to detect contamination of filters used in the field. There are many opportunities for contamination to occur, for example, during assembly, calibration, or storage. Therefore, each investigation should be carefully monitored by including 10% field blanks or 2 filters minimum. However, since these blanks are not specific, other kinds of blanks are necessary to pinpoint the exact source of the contamination. Field blanks also provide quality control for the gravimetric data.
3. **Filter blanks** are used to validate filter cleanliness prior to calibration, as well as, to check the sterile technique of the technician. At least one filter blank should be made for each batch of filter cassettes that are assembled, including batches that are sent out for sampling.

Bulk Sample Collection:

1. Collect samples in endotoxin-free vessels (glass scintillation vials baked as provided in the glassware protocol).
2. Liquid samples may be collected by immersion, capture of a stream from spouts, or by use of sterile individually-wrapped pipettes.

3. Collect solid bulk samples using endotoxin-free forceps, or using a standard vacuum cleaner protocol that allows samples to be transferred to the collection vessel without contamination by the technician's skin flora.
4. Cover the glass vial with the clean side of a piece of parafilm. Screw on the vial cap until seated in the parafilm without cutting through it.
5. Pack samples in plastic bags surrounded with blue ice.
6. Ship samples by over night carrier to the analytical laboratory.

Labeling and Record Keeping

1. Assign all filter cassettes a unique number at the time the cassette is prepared. Numbers will composed of the letter A, followed by a letter (A through L) for the month, a 2 digit number (01 - 31) of the day's date, and a number for the last digit of the year. Finally the cassette ID will end with a dash and the number of the filter for the day. If the filters are not to be pre and post weighed, add a suffix of U to the cassette number.
2. When the sampling data sheets will be kept with the filters during storage until the sample is post weighed (or assayed if not post weighed). During the post weighing, the cassette will be labeled with the pump-off date and the sample location ID.
3. Notebook entries for the assays will record the filter cassette ID, the pump-off date, and the sample location ID.
4. Cassettes prepared for outside labs will not be given a cassette number unless pre weighed. Notebooks will record the outside lab or source of sample and the cassette number.

GLASSWARE AND UTENSILS

Washing:

1. Clean soiled reusable glassware and metal utensils: soak in a dilute detergent solution and wash as needed. Then, rinse twice with tap water and two more times with distilled water, and air dry. If the vessel was used only with buffer or water, a distilled water rinse is sufficient. (Reusable glassware includes Pyrex or Kimax graduated cylinders, flasks, beakers, and bottles. Metal utensils include spatulas, forceps, inoculating needles, and steel cassette screens.)
2. New disposable glassware including borosilicate glass scintillation vials, 12 X 75 mm and 13 X 100 mm test tubes, Pastuer pipettes do not need to be washed. They must be inspected for cleanliness and baked to ensure that that they are endotoxin-free. Sterile, individually wrapped pipettes are used "as is" from the manufacturer without any additional treatment. These items are usually endotoxin-free.

Wrapping:

1. Place a double layer of aluminum foil on the openings and exposed surfaces of glassware.
2. Load disposable test tubes open end down into an appropriately sized beaker, and cover the top of the beaker with a double layer of aluminum foil.
3. Wrap metal utensils in foil to obtain at least a double layer of foil.

Baking:

Place prepared items in an oven and bring the oven temperature to 270°C +/- 5°C. Bake for 30 minutes. Remove when cool and stock in a clean dry location.

TRIETHYLAMINE PHOSPHATE (TAP) BUFFER

Phosphate Stocks:

Weigh out salts:

STOCK A Dibasic: (1.0 M K_2HPO_4 Formula Weight = 174.18 g)

Weigh out 17.42 g and place in an endotoxin-free 100 ml bottle.

STOCK B Monobasic: (0.2 M KH_2PO_4 Formula Weight = 136.06 g)

Weigh out 2.72 g and place in an endotoxin-free 100 ml bottle.

Bake out salts:

Cover the tops of the 100 ml bottles with a double layer of aluminum foil. Turn each bottle on its side and lightly shake so the salt is uniformly distributed across the side of the bottle. Place prepared bottles in an oven and bake at 180°C for four hours +/- 5°C. NOTE: Make sure that the temperature does not exceed 185°C; salts become insoluble if they are bake out at higher temperatures. Remove the salts from the oven and allow them to cool.

Dissolve salts:

After the salts have cooled, transfer the bottles to a biological safety hood to prevent airborne contamination. Label the bottles with date made and initials of the technician. Remove the foil coverings and add 100 ml of sterile water (e.g. Baxter cat # 2F7114) to each bottle. Cover with parafilm and swirl occasionally, without touching parafilm, and allow to sit for approximately 2 h to dissolve salts. Refrigerate until needed.

Triethylamine, 1% (TEA) Stock:

Label an endotoxin-free bottle with date, and initials of the technician. Add 99 ml of sterile water (e.g. Baxter cat # 2F7114). Add one ml of triethyamine (Fisher cat # 04884-100). Cover with parafilm and agitate vigorously taking care not to let solution come in contact with parafilm. Refrigerate until needed.

Working Buffers:

Label endotoxin-free bottles including a buffer number, date, and initials of the technician. Add 3.9 ml Stock A, 5.1 ml Stock B, 90 ml of pyrogen-free water (Baxter #2F7114), and 1.0 ml 1% TEA Stock. Record appropriate information in lab book (i.e. stocks used, open date of pyrogen-free water used).

Quality Control and Record Keeping:

1. Test each bottle of buffer, prior to use, to ensure that pH is 7.50 +/- 0.04 using a pH meter by placing two one milliliter aliquots of buffer in 12 X 75 mm tubes. Calibrate the pH meter using standards at pH 7.0 and 10.1 prior to reading pH for the buffer samples. Record pH of each aliquot in the lab book.
2. Perform Limulus assay of each bottle of buffer, Limulus reaction water (LRW), and Baxter pyrogen-free water to ensure that they are endotoxin-free before use. Follow the KLARE Protocol with the following modifications. Instead of making serial dilutions, divide the 96-well plate into four quadrants and add an undiluted 50 µl aliquot from each buffer to each of the four quadrants (a total of four aliquots from each buffer will be added to the plate). Buffers are acceptable if after a 120 min incubation the final absorbance is < 100 mOD and if the Vmax (rate) is < 1 mOD/min in at least 3 of the test wells. For LRW and Baxter sterile water the upper limit of acceptable Vmax is 5 mOD/min.
3. It is essential to track each bottle and lot of every component of the buffer so that it can be readily identified when buffers and stock solutions have expired. Therefore, it is important to label each bottle with the date it was made and the date it was first opened. It is also essential to record the stock solutions used in making each buffer, and to record what bottle of buffer (diluent) was used in each assay.

SAMPLE EXTRACTION

Preparation of Filter Samples:

1. Remove filters from cassettes: Grasp cassette in one hand and hold firmly. Open scissors and use one inside edge to slowly pry apart the cassette between the bottom and middle pieces. Work your way around the cassette gently and evenly until you are able to pull it apart by hand. DO NOT OPEN YET!
2. Assess the appearance of the filter and record in notebook: Filter appearance varies from sample to sample and is used as a rough indication of endotoxin content. Filters that have little or no sample visible are identified as "clean". Filters that have sample visible only in the center of the filter are identified as "dirty in center". Filters that have sample visible on the entire surface of the filter are identified as "dirty over entire". Gradations within these categories as well as color notations may also be noted (i.e. slightly dirty in center, black over entire).
3. Extract all filters in 5 ml of TAP buffer. Use the appearance of the filter to determine the initial dilutions for the KLARE protocol. Assign filters an initial dilution factor of one if they are "clean," background samples, or from office or domestic environments. Assign an initial dilution factor of 6 to filters that are dirty over the entire surface of the filter. The initial dilution factors can be increased if specific groups of samples appear to warrant it (i.e. filters which sampled metal working fluids).
4. Transfer filter to extraction tube: Label an endotoxin-free 13 X 100 mm tube with sample ID. Open cassette and grab filter at edge using endotoxin-free forceps. Keep the filter face up at all times and keep motion to a minimum so no material is lost from face of filter. Grab opposite edge with another pair of endotoxin-free forceps, and gently roll filter (make sure the filter sampling face is on the inside) around forceps until it is small enough to fit into the labeled tube. Place filter in the tube. Cover the tube with parafilm. Analyze immediately or store in an air tight container with desiccant at 4°C. A pin hole may be punched through the parafilm to allow air circulation.

Preparation of Dry Bulk Samples:

Label an endotoxin-free 13 X 100 mm tube with sample ID. Using endotoxin free utensils and sterile technique, weigh out an appropriate quantity (e.g. 25 mg of sifted house dust (400μm screen)). Record mass of sample. Cover the tube with parafilm. Analyze immediately or store desiccated at-20°C in airtight container as described for filters.

Addition of Extraction Buffer to Filter and Dry Bulk Samples:

Add 5 ml of TAP buffer to each of the filter and dry bulk samples using a fresh, sterile, ten milliliter pipette. Change the pipette after every second sample or more frequently if contamination of the pipette tip is suspected. Cover with parafilm and gently vortex filter samples for 10 seconds. Gentle vortexing is necessary to prevent the filter from collapsing

into a pellet in the tube. Vortex bulk sample as vigorously as possible to break up any clumps without bringing the fluid into contact with the parafilm. Check to see that bulk dust and filters are entirely submerged. If necessary, vortex again. If the filter its not submerged after vortexing, shake filter down to the bottom of the tube by giving it a gentle but swift snap of the wrist.

Preparation of Liquid Bulk Samples:

1. Liquid samples should be analyzed within 24 hr of arrival in the laboratory. They may be stored undiluted at 4°C until analysis. Freezing may result in significant loss of endotoxin activity, especially for metal working fluids and other surfactant containing materials.
2. Label an endotoxin-free 13 X 100 mm tube with sample ID.
3. Transfer a defined volume of the sample into the extraction tube and add an appropriate volume of TAP buffer to a final volume of 3 to 5 ml. Cover the tubes with parafilm and vortex for 10 seconds.
    a) Samples which may contain significant amounts of endotoxin should be extracted after dilution of 5 to 100 fold in buffer. Refer to the Table of Standard Dilutions for dilution recipes.
    b) When measurement of low endotoxin concentrations is desired it may be necessary to process samples without extraction in buffer. However, extraction in buffer should be used when possible to provide uniform extraction conditions.

Bath Sonication:

Place test tubes in rack and place in a bath sonicator (i.e. Bronson 3200) filled within one inch of the top of the test tubes. Sonicate 60 minutes. Samples should be vortexed every 15 minutes (t = 0, 15, 30, and 45 minutes) during extraction. Maintain constant bath temperature using a cooling coil.

Extract Completion:

1. Remove tubes from bath at t = 60 minutes and thoroughly dry the test tubes.
2. Bulk samples are ready for the Assay Protocol. Fine dust particles are not removed from the extracts prior to assay as these may continue to have surface associated endotoxin.
3. Vortex filter samples vigorously to pellet the filter. If it is necessary to separate the filter and fluid, use a sterile pipette to transfer the fluid to another tube. Filter extracts can now be analyzed using the Assay Protocol.

ASSAY PROTOCOL

Materials

200 µl Automatic Pipette and Endotoxin-free Tips
1000 µl Automatic Pipette and Endotoxin-free Tips
Beakers (for waste)
Bucket Filled With Ice
Control Standard Endotoxin (CSE, Associates of Cap Cod)
Ethyl Alcohol, 75%
Incubated Microplate Reader
LAL Reagent Water (LRW from Associates of Cape Cod)
Laminar Flow Hood
Limulus Assay Lysate: KQCL (LAL, BioWhittaker)
Metal Block for Microplate (Cooled to -20°C)
Microplate (96-well Pyroplate, Associates of Cape Cod, or glass microplate, BioWhittaker)
Microplate Reader With Incubator
Parafilm
Pipettes (5 ml, individually wrapped, glass)
Pipette Bulb
Reference Standard Endotoxin (RSE, United States Pharmacopoeia)
Rotary Shaker
TAP buffer
Test Tubes (12 X 75 mm, endotoxin-free, borosilicate glass)
Test Tubes (13 X 100 mm, endotoxin-free, borosilicate glass)
Test Tube Racks
Vortex Mixer

Overview:

Endotoxin or lipopolysaccharide (LPS) is an amphiphatic molecule found in the outer membrane of Gram-negative bacteria. The lipid portion of LPS (lipid A) is chemically distinct from all other lipids and is responsible for the characteristic toxicity of LPS.

One of the major analytical methods used to determine the presence of endotoxin is the *Limulus* amebocyte lysate (LAL) test. Improvements in *Limulus* reagents allow their application in quantitative assays. *Limulus* based methods are in vitro biological assays in which LAL is activated in the presence of endotoxin. In the chromogenic method, once activated, the LAL enzymes hydrolyzes a synthetic substrate causing the release of a chromophore which is detected spectrophotometrically at 405 nm.

Limulus assays are comparative bioassays. They are not analytical assays. This means that factors other than changes in the concentration of LPS can affect the measurement result. Therefore, strict control of the assay conditions is essential to achieve useful and comparable measurements.

Sample collection, glassware and buffer preparation, and sample extraction are described in separate protocols. Briefly, samples are stored desiccated at 4°C until assay. In preparation for the assay, samples are extracted in 5 ml of a triethylamine phosphate (TAP) buffer. After extraction, filter extracts (extraction fluid without filter) and bulk extracts (extraction fluid with dissolved or suspended bulk sample) are serially diluted in endotoxin-free test tubes. Duplicate aliquots from each tube are placed in a polystyrene microplate and mixed with LAL at 4°C. Serial dilutions of the standard, sample, and negative controls are assayed together in the same 96-well microplate. Each well is monitored every 30 seconds for a period of 120 minutes at 405 nm during an incubation at 37°C. The standard and sample dilution curves generated by the reaction rates in the wells are compared using the method of parallel-line bioassay analysis to determine the validity of the assay, and to determine endotoxin concentrations of the unknowns.

Assay Set-up:

1. Make sure there is plenty of endotoxin-free glassware available.
2. Turn on the computer and plate reader, checking that the temperature is set to 37°C, one hour before you plan on placing a microplate in the reader. Turn on the laminar flow hood 15 minutes before use. Wipe down the interior of the hood with 75% ethyl alcohol.
3. Fill ice bucket; keep standard, LAL, LRW and other samples refrigerated or on ice at all times except during use. This extends the life of the materials and maintains positive and negative controls.
4. Design dilution schedule in notebook and make template of dilutions and microplate layout to be referred to during placement of the samples in the microplate. Refer to the Table of Standard Dilutions and the Standard Horizontal Layout for information on dilution recipes and proper placement of samples.
5. Place two beakers inside the laminar flow hood to be used for waste. This procedure makes it easier to work inside the confined space of the hood and minimizes the amount of time spent with hands outside the hood or over the test tubes.
6. For the Control Standard Endotoxin (CSE) and each unknown, place five endotoxin-free 12 X 75 mm test tubes in racks inside the hood (NOTE: If you are using Reference Standard Endotoxin (RSE) you will need an initial dilution factor of 100. Therefore, use an endotoxin-free 13 X 100 mm test tube for the first of the five dilution tubes.) Group the tubes in distinct sets of five adjacent tubes with the initial dilution tube in the left most position of the group. The remaining tubes should be placed in left to right order of increasing dilution (i.e. 5th dilution tube in the right most position). This reduces confusion while the serial dilutions are performed. Refer to the dilution schedule.
7. Using a 1000 µl automatic pipette and polypropylene tip, place the appropriate amount of diluent (TAP buffer) in each tube according to the dilution schedule. Care must be taken in order to ensure that the pipette tip only comes in contact with the inside of the TAP stock bottle and the inside of the dilution tubes. If the tip touches any surface

other than these areas, discard the tip and continue on with another. Apply the same philosophy whenever you work with pipettes during the course of the assay. As long as the tip remains clean, the same pipette tip can be used to deliver diluent to the standard and sample tubes.

8. Prepare negative controls using a fresh pipette tip for each fluid, place one milliliter of TAP buffer in one tube, and one milliliter of LRW in another.
9. Prepare the sample extracts as outlined in the Sample Extraction Protocol. Place the sample extracts behind the appropriate sets of dilution tubes. Refer to the microplate layout to verify the correct placement of extract tubes. Record information for the assay on the KLARE assay information sheet.

Serial Dilution:

1. Before making the initial dilution of the endotoxin standard, vortex the vial of standard for ten seconds using extreme care. While vortexing, prevent the standard from coming in contact with the parafilm covering the vial. If RSE is used, gently roll or shake the vial by hand to suspend the endotoxin. Remove the parafilm. Using an automatic pipette, set to the volume determined by the dilution schedule, and a 200 µl polypropylene pipette tip draw up and discard the first volume of standard. Draw up and deliver the second volume of standard to the first dilution tube for the standard. Vortex the initial dilution for ten seconds. NOTE: For any amount of standard or sample 50 ul or less, the first aliquot drawn up in the pipette tip is always discarded. This coats the pipette tip with the liquid and ensures a more accurate delivery of subsequent volumes. Recover the standard vial with parafilm (clean side facing vial opening) and return it to the ice bucket.
2. Reload the pipette with a fresh tip and proceed to make the initial dilution of the first unknown following the protocol described for the standard. Continue until the initial dilutions are complete for the standard and all of the unknowns. Then, begin with the standard and make the second dilution in the series for each of the samples. Proceed with each sample in the same manner as for the initial dilution. Work at a steady pace and perform each of the serial dilutions in the same order as that of the initial dilution step (i.e. standard, sample A, sample B, etc.).
3. It is essential to vortex all source test tubes before and all receiving tubes after transferring volumes. This is especially important for particulate samples since they tend to settle to the bottom of the tubes in a relatively short amount of time. When withdrawing aliquots from the standard vials and sample extraction tubes, tip them so that the liquid is at the lip of the vessel, then withdraw an aliquot. This reduces the risks of contamination by preventing dirty pipettes from coming in contact with standards and samples, and by limiting the amount of time working directly over the surface of the exposed liquids.

Method for Loading Microplates:

1. When the dilutions are completed, remove a microplate from its wrapper inside the hood. Place the lidded microplate in a metal block cooled to -20°C and prop the block up on the lid of a 1000 µl pipette tip box. This part of the assay requires a great deal of concentration. It is not always possible to tell that a well is loaded from further tipping the plate to look in the wells. Do not rush.
2. Refer to the "Standard Horizontal Layout" for proper placement of samples. Record placement of samples in the microplate on the "KLARE Assay Information Sheet" in the "Horizontal Layout".
3. Begin adding standards and samples to the plate by starting with the highest standard dilution tube. Add 50 µl from each dilution tube to duplicate wells using one pipette tip for every tube. Discard the first 50 ul aliquot, load the second and third aliquots into the microplate. After all samples have been placed in the plate, load the LRW and buffer blanks; five wells of each should be loaded, using one pipette tip for LRW, and a second pipette tip for the buffer blank. Discard the first 50 ul and load the subsequent aliquots. Replace the microplate lid.

Rehydration and Addition of BioWhittaker LAL:

1. Take the bottle of LRW and two bottles of LAL from the ice bucket and put them in the laminar flow hood. Using a 1000 ul pipette tip, aseptically remove the rubber stopper located under the metal cap on each of the two LAL vials. NOTE: If there is no noticeable vacuum on an LAL vial, discard it and replace it with another. Fill a sterile five milliliter pipette with 5.2 ml of LRW using a pipette bulb. Release 2.6 ml into each of the LAL vials. Carefully tip the LAL bottles so that the liquid inside approaches the inner lip of the bottle. Gently turn the bottle so that the LRW completely coats the inside of the vial (this dissolves the LAL sticking to the sides of the vial). Allow rehydrated LAL to stand in the hood for five minutes.
2. After five minutes, carefully tip and turn each vial so that the inner surface of each vial is rinsed. Visually inspect the fluid in each vial to ensure that the liquid is clear and colorless. If the liquid is yellow in color or has noticeable particulates, discard and replace it with another.
3. Place a repeating pipette tip on the pipettor using sterile technique.
4. Transfer the contents of one LAL bottle to the other bottle using the repeater pipette. Be extremely careful that the pipette tip only touches the surfaces inside the vials. Mix the combined LAL by drawing up another pipette full of the fluid and dispelling it back into the vial. NOTE: Transfer and mix the LAL solution gently so that no foaming occurs.
5. Make sure the pipette is set to deliver 50 µl volumes and refill the pipette. Expel the first 50 ul aliquot back into the vial. Start by filling wells in column 6 containing buffer and LRW negative controls. Expel 50 µl of LAL into each well. Then, continue expelling aliquots of LAL into the sample and standard dilution wells on the left side of

the plate beginning with column 5 (lowest concentration samples) and proceeding to progressively more concentrated samples. Continue snaking up and down the columns until the last aliquot is expelled in the last well of the first column. (The preferred order starts at the bottom of column 5 and ends at the top of column 1.) Discard the remaining LAL and discard the pipette tip.

6. Replace the repeater pipette tip and fill it with LAL. Expel the first 50 µl aliquot back into the vial. Expel 50 µl aliquots into the LRW and buffer blank wells in column 7. Then, continue expelling LAL aliquots starting with the lowest concentrations on the right half of the microplate (i.e. bottom well of column number twelve). Proceed up column 12 and down column 11 and so on until you've reached the top of column 8. Discard tip. (Note: It is important to add LAL quickly yet gently in order to prevent splashing. Also, the tip should not come in contact with fluid in any well since subsequent wells may thus be contaminated. This part of the assay requires maximum concentration.)

Transfer to Microplate Reader and Kinetic Absorbance Measurement:

1. Once LAL is added, replace the microplate lid and gently remove the plate from the hood to a rotary shaker. Shake the microplate for 10 seconds on low setting. If the shaker is not equipped to fasten the microplate, hold it on the shaker with one or two fingers on the lid. (NOTE: If the shaker is turned up too high, the contents of the wells will splash on the lid. Thus, it is important to know the proper setting in advance.)
2. Place the plate in an incubating kinetic microplate reader set to maintain a temperature of 37°C. Remove the microplate lid. Program the reader for an initial shake of 30 seconds and a monitoring interval of 30 seconds for a period of two hours.
3. Record kinetic absorbance data.

NOTE: The most important aspect of this assay is that it is performed consistently. The amount of time samples are vortexed must be as consistent as possible. The rate at which LAL is added to the microplate should also be steady and consistent.

KLARE DATA ANALYSIS:

1. From the absorbance data for each microplate well, determine the maximum rate of optical density change (Vmax, mOD/min) during the 2 h incubation.
2. Compute a dose-response curve for the standard endotoxin from the Vmax and log endotoxin concentration data using least-squares or resistant regression methods. Determine whether the slope for the standard curve falls within quality control limits for the LAL lot and standard in use. This may vary between microplate readers and lots of LAL. If the standard slope is out of range, repeat the assay.
3. Compare the dose-response of each unknown sample with that of the standard using analysis of covariance.
4. Examine data for dose-dependent interference if a dose-response curve for an unknown shows a low probability of being parallel to the standard curve ($p < 0.05$). Eliminate high concentration points on the unknown curve from the data analysis and repeat the analysis of covariance. If the unknown sample continues to fail a test for parallelism with the standard after elimination of half of the observed dilutions, then the analysis of this sample is invalid (dilution independent interference) and the sample must be reanalyzed.
5. Compute the log potency ratio and its standard error using the single slope, separate intercepts regression model using least-squares or resistant regression. Use the largest number of observations on the unknown that resulted in a line parallel with the standard.
6. Compute the potency and its 95% confidence interval from the log potency and standard error.
7. Convert units of control standard endotoxin to Endotoxin units with reference to EC6 (or the current USP standard). This conversion requires knowing the potency of the CSE compared with the potency of EC6 when diluted in TAP buffer and assayed in the same lot of LAL used to assay the unknown samples.

Computer software for KLARE data analysis is available from the Environmental Endotoxin Laboratory, Occupational Health Program, Harvard School of Public Health. The software supports a variety of microplate readers including those marketed by BioWhittaker, BioTech, Molecular Devices, and others. A user manual and documentation for the software will be available in the future.

CONDITIONS OF ANALYSIS BY HARVARD ENDOTOXIN LABORATORY:

1. The Harvard Endotoxin Laboratory is primarily a research laboratory. Samples can be analyzed on a fee for service basis as these fit into our research schedule and particularly as they contribute to our ongoing effort to characterize endotoxin exposure in a variety of industrial and other environments.
2. Participants in these investigations agree to allow use of data and samples for research purposes including scientific publication. The endotoxin laboratory will not use location or corporate identifiers in such publications unless specifically authorized by the participants.
3. Participants agree to provide bulk samples where appropriate.
4. Participants must provide descriptions of the jobs and processes involved that are scientifically useful without jeopardizing proprietary information.

Protocol and Quality Assurance Plan for Nicotine Analysis

Protocol and Quality Assurance Plan for Nicotine Analysis

S. Katharine Hammond, Ph.D.
June 14, 1999

Equipment:

Gas chromatograph, Hewlett Packard 6890, Auto sampler HP18596C
Gas chromatograph, Hewlett Packard 5890, Auto sampler HP7673A
Both equipped with nitrogen phosphorus detector (NPD)
Precision sampling syringe, 10 ul reinforced plunger
Vortex mixer
Adjustable volumetric pipettes: (1 ml aqueous Pipetman; 1 ml and 250 ul positive displacement)

Chemicals:

Absolute ethanol, reagent grade
Sodium bisulfate, monohydrate, reagent grade
Nicotine (Kodak chemical #1242)
Heptane Baker-Analyzed for hplc
Distilled, DI water
Sodium Hydroxide pellets, reagent grade
Gases: Helium (99.995 % purity), Ammonia (anhydrous)
Air (zero grade), Hydrogen (zero grade)

Supplies:

Filters, teflon coated glass fiber (Pallflex
TX40H12 WW)
Sample vials (New): 2ml, 12x32mm, clear glass
350 ul glass inserts
Watch glasses
Aluminum foil
Tweezers
13x100 mm borosilicate glass disposable test tubes

Preparation of reagents:

1. Solution for treating filters--Prepare weekly
4% by weight sodium bisulfate in water 5% ethanol by vol., 2% glycerol by vol.

Dissolve 4 g sodium bisulfate in water
Add 5 ml ethanol, 2 ml glycerol
Dilute to 100 ml with water

2. Extraction Solutions

a. 5% ethanol in water - prepare daily
Dilute 5 ml of ethanol to100 ml with distilled, DI water

b. 10N NaoH
Place 80 grams NaoH pellets in a 500 ml erhlenmeyer flask
Dilute to 100 ml with water

c. Ammoniated heptane - prepare daily
Bubble ammonia (in hood) through 100 ml of heptane for 2 min. to saturate

3. Standard Solutions

a. Nicotine Stock Solution—1000 ug/ml
Prepare fresh every month wearing gloves
Add about 50 ml ammoniated heptane to a 100 ml volumetric flask
Measure 100 ul of nicotine with a syringe and add to heptane -
Swirl to mix first
Dilute to mark with heptane
Mix well
Place aliquots into five 10ml crimp top vials and seal.
Label each vial with concentration, date, and initials
Store in freezer
Open a fresh vial each week to prepare standards
Reseal immediately after use and replace in freezer

b. Standard Solutions
Use 1 ml Pipetman pipet with fresh tips for each solution
All dilutions are made with ammoniated
heptane in volumetric flasks to mark.
100 ug/ml stock nicotine solution
Prepare fresh daily
Pipet 1 ml of the 1000 ug/ml mg/ml stock solution into a 10 ml volumetric flask
Dilute to mark with ammoniated heptane

Analysis Standards
Standards can be stored in freezer for a week
Standards each day should be over at least a tenfold range of concentration.
Prepare from 100 ug/ml stock (see next page for dilutions)

Standards:

Initial conc. ug/ml	Pipet amount ml	Final conc. ug/ml	Vol. flask ml
1000	1	100	10
100	1	10	10
100	1	4.0	25
10	1	2.0	5
10	1	1.0	10
4.0	1	0.4	10
1.0	1	0.2	5
1.0	1	0.1	10
0.4	1	0.04	10
0.1	1	0.02	5
0.1	1	0.01	10
0.04	1	0.004	10

Preparation of Treated Filters:

Fill watch glass with sodium bisulfate treating solution. With tweezers, place a fresh filter on the surface of the liquid. It should be wetted immediately.

Analysis Procedures:

1. Daily prepare standard nicotine solutions from stock nicotine solutions expected range of sample. Standards should be at least over a tenfold range of concentration and bracket all samples. Inject 3 ul to establish linearity within that range. Make duplicated injections to check reproducibility. Injections should agree within 5%. The correlation coefficient of the line should be at least 0.9997.

2. Prepare two or three spiked filters at level of samples.
   For example, Pipet 10 ul of 100 ug/ml standard onto a Treated filter for a 1ug spike.
   Extract as samples (see below).
   Extract the spikes and one blank filter as outlined below.
   Inject into the GC and quantitative.
   Recovery should be at least 90% +-3%. The recovery from the spikes must be satisfactory before proceeding with sample preparation.

3. Extraction of nicotine from treated filters
   1. Transfer filter to test tube.
   2. Add 2 ml 5% ethanol solution and vortex 1 minute.
   3. Add 2 ml 10N NaoH
   4. Add 250 ul, 2 times of ammoniated heptane (measured with positive displacement pipet) and vortex 1 minute.
   5. Draw off top layer of prepared sample and transfer to reduced volume vial. Cap with crimp top.
   6. Load into auto sampler or inject manually.

4. Duplicate injections of all samples required.
   Intersperse the samples with standards so that there are no more than six samples between standards.

5. At the end of the day's run, change the septum on the GC injector and check flow rates of all gasses.
   This procedure should be carried out in an environment free from ambient nicotine to prevent contamination of the treated filters.

Instrument Settings for the 6890:

Bead: Under the Instrument pull-down, select Edit Parameters, then select Detector Select Adjust, enter 60 for the offset, and 3.00 minutes for the equilibration time Allow the detector to adjust the offset (should last between 5 and 20 minutes)

Oven:

Initial temp:	90 C
Initial time:	0 min

Ramps:

#	Rate	Final Temp
1	14 deg/min	130
2	30 deg/min	220

Run time: 5.86 min

Front Inlet:

Mode:	Pulsed splitless
Initial temp:	250' C (ON)
Pressure:	8.5 psi (ON)
Pulse pressure	50 psi
Pulse time:	0.75 min
Purge flow:	50.0 ml/min
Purge time:	0.75 min
Total flow:	55.4 ml/min
Saver flow:	20.0 ml/min
Saver time:	2.00 min

Column 1:

Capillary Column:	Cross linked 5% PH ME Siloxane
Model number:	J&W DB - 5
Max temperature:	320 ' C
Nominal length:	15.0 m
Nominal diameter:	320 um
Nominal film thickness:	0.25 um
Mode:	Constant flow
Initial flow:	3.0 ml/min
Column pressure:	8.5 psi
Average velocity:	56 cm/sec

Front Detector (NPD):

Temperature:	250 C
Hydrogen flow:	3.0 ml/min
Air flow	60.0 ml/min
Mode:	constant column+makeup flow
Makeup flow:	6.7 ml/min
Gas type:	Helium
Adjust offset:	60.00
Electrometer:	On
Bead:	On
Equilibration time:	0.10

7673 Injector:

Injection Volume:	3.0 microliters
Syringe Size:	10.00 microliters
Nanoliter Adapter:	Off
Viscosity Delay:	0 seconds
Plunger speed:	Fast

Set Auto Sampler to:
Wash 5 times with sample (placed in numbered vials)
Pump 5 times with sample
Inject 3 ul
Wash 7 times with isopropanol and 7 times with heptane

Integration Event Table "Event NPD1A":

Event	Value	Time
Initial Area Reject	0.000	Initial
Initial Threshold	0.010	Initial
Initial Peak Width	0.010	Initial
Initial Shoulders	Off	Initial
Integrator Off		0.000
Integrator On		2.700
Baseline Now	0.000	2.700

Apply Manual Integration Events: Yes

Specify Report:

Add Chromatogram Output:	Yes
Chromatogram Output:	Portrait
Size in Time direction:	100%
Size in Response direction:	20% of page

Instrument Settings for the 5890:

Analytical conditions: GC-NPD
Column: 10% apiezon L, 3% KOH on WHP 80/100, 8' length

GC Keyboard:

Temps: oven temp: 225 C
Injector temp: 250 C
Detector temp: 300 C
(keep detector at 100-150 C when system down)
Adjust the signal to between 24 and 50 picoamps by setting the bead voltage.
Bead voltage range: 40-60 (usually 40)

Gas Flows:

Helium: 30 ml/min (70 psi @ tank) (32 psi @ room T) 50 psi
H2: 3-5 ml/min (20 psi @ tank)
Air: 120 ml/min (50 psi @ tank)

Set Auto Sampler to:

Wash 5 times with sample (placed in numbered vials)
Pump 5 times with sample
Inject 3 ul
Wash 7 times with isopropanol and 7 times with heptane

Integration Event Table "Event NPD1A":

Event	Value	Time
Initial Area Reject	0.000	Initial
Initial Threshold	0.010	Initial
Initial Peak Width	0.010	Initial
Initial Shoulders	Off	Initial
Integrator Off		0.000
Integrator On		2.700
Baseline Now	0.000	2.700

Apply Manual Integration Events: Yes

Specify Report:

Add Chromatogram Output:	Yes
Chromatogram Output:	Portrait
Size in Time direction:	100%
Size in Response direction:	20% of page

Software Procedures:

In HP group select Instrument 1 Online for 6890 or Instrument 2 Online for 5890
Load NIC method for 5890 or 6890
Under the "Sequence" pull-down, select Parameters
Create a new subdirectory name based on date/sample source
Exit Parameters
Under the "Sequence" pull-down, select Table
Fill out the table using Vial numbers and Sample names which correspond to positions in the autosampler tray, use Method Name NIC, and select the number of desired injections for each sample
The spaces under Cal line, Sample Amount, and ISTD amount can be left blank
Exit table
Save the Sequence using a name based on date/sample source
Under the "Run Control" pull-down, select Run Sequence
The table can be edited during a run, however the sequence must be saved again after editing
To view the integration results during a run, select data analysis under the "View" pull-down

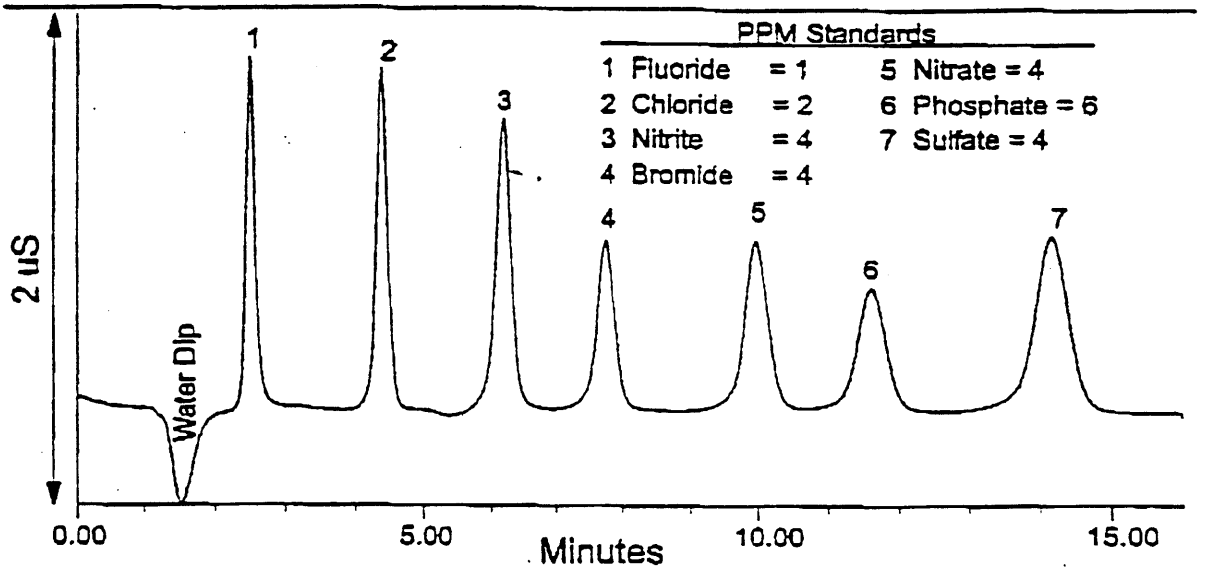
Waters Ion Chromatography Method

Waters

Ion Chromatography Method

General Anion Analysis Using Chemically Suppressed Conductivity Detection
Equivalent to EPA Method 300.0 1998

Required Instrumentation:	Part / Number
Alliance, 2690 Separations Module (with Column Heater, Seal Wash, Degasser)	271013
432 Conductivity Detector	043061
Bus SAT/IN Module	073645
Millennium, Ver 2.15.01, or Millennium 32	Consult Waters
Alltech ERIS™ 1000HP Autosuppressor	Consult Alltech



Analysis Conditions:

Column:	IC-Pak Anion HR
Eluent:	1.6 mM $NaHCO_3$ / 1.4 mM Na_2CO_3
Back Conductivity:	10-15 µS
Regenerant:	DI Water or eluent
Degas:	Continuous
Flow Rate:	1 mL / min
Back Pressure:	1250 ± 200 psi
Temperature:	30°C (Column Heater); 35°C (Detector)
Injection:	25 µL
Needle Wash:	12% AcCN in DI Water
Detection:	Chemically Suppressed Conductivity
Base Range:	20
Attenuation:	100 µS / Volt
Polarity:	Positive

Eluent Preparation:

1) Into a 1 liter volumetric flask add
   -16 mL of 100 mM $NaHCO_3$
   -14 mL of 100 mM Na_2CO_3
2) Dilute to volume with DI Water
3) Vacuum degas through a 0.45 µm aqueous compatible membrane
4) Store in a glass or plastic container at ambient temperature. Discard after 1 week.

Standard Preparation:

It is recommended that certified 1000 ppm anion standards be used with this method. If unavailable, see Reagent Section for uncertified standard preparation.

Prepare at least 3 mixed analyte standards within the expected range of the sample analyte concentration. This method is linear from 0.1 ppm to 100 ppm. After the multi-point calibration curve has been validated, a single point calibration within the expected analyte concentration is appropriate for future calibrations.

Sample Preparation:

Determine the expected range of analyte concentration and other anionic component in the sample matrix. The major analyte should be less than 100 ppm for best results.

If necessary dilute the sample with DI Water.

If the sample contains high amounts of neutral organics, or is highly colored, then pass the diluted sample through a C_{18} Sep-Pak Cartridge. Anions pass through unretained, but may note a loss of fluoride recovery.

Samples containing suspended solids should be filtered through a 0.45 µm aqueous compatible disk prior to injection. Failure to filter solids results in the risk of increased column backpressure.

Sample pH should be within 3 to 11 for best results.

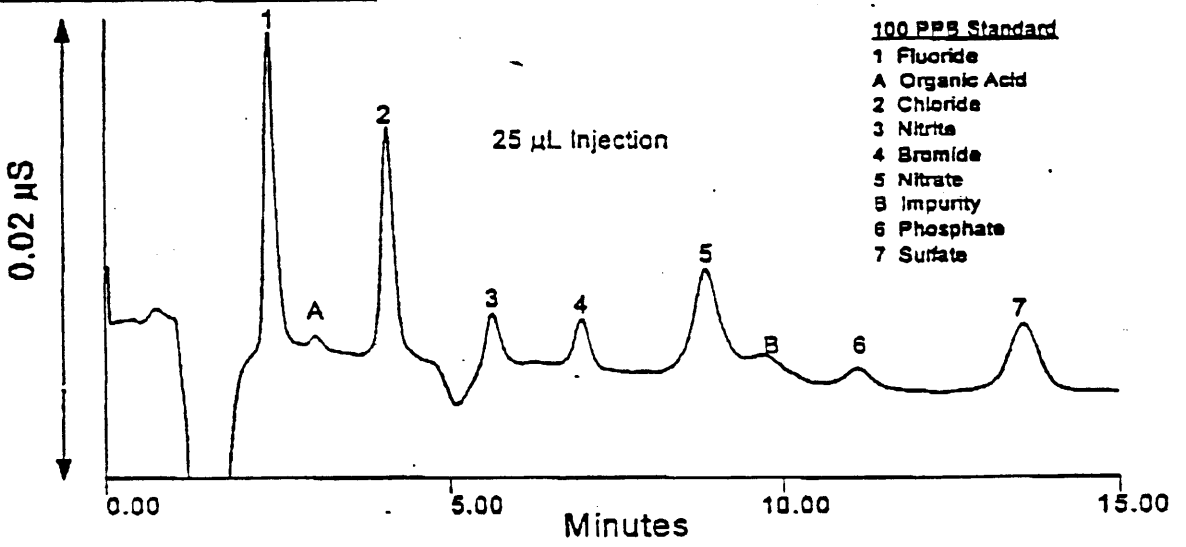
To minimize the water dip and improve quantitation of fluoride, dilute with eluent.

Millennium Data Processing Method:

IC Processing Method using Peal Apex for Retention Time

Integration	Peak Width	= 30.0	Threshold = 4–8
	Min Area	= 1500	Min Height = 75
	Inhibit Intg.	= 0 to 2 min	
Calibration	Averaging	= None	RT Window = 5%
	Update RT	= Never	
	Peak Match	= Closest	
	Quant By	= Peak Area	
	Fit Type	= Linear, for multi-point calibration or Linear Through Zero, for single point	
Report	Analyte Name		
	Retention Time		
	Peak Area		
	Amounts		

Method Detection Limits:



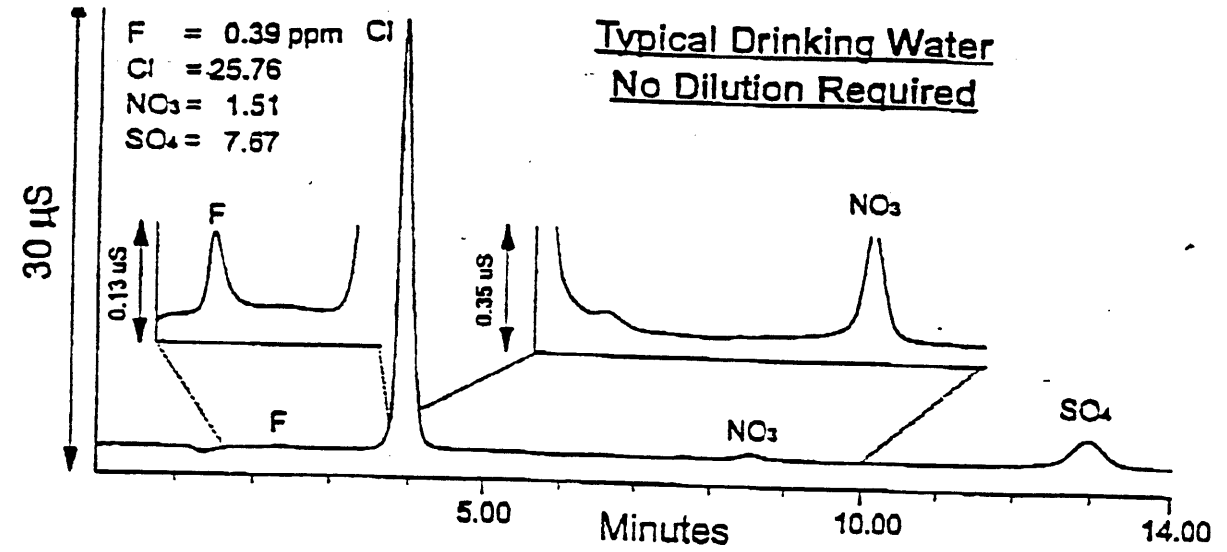
Based upon this representative chromatogram using a 25 µL injection, the estimated detection limits, as ppb, at 3 times signal to noise (S/N) are:

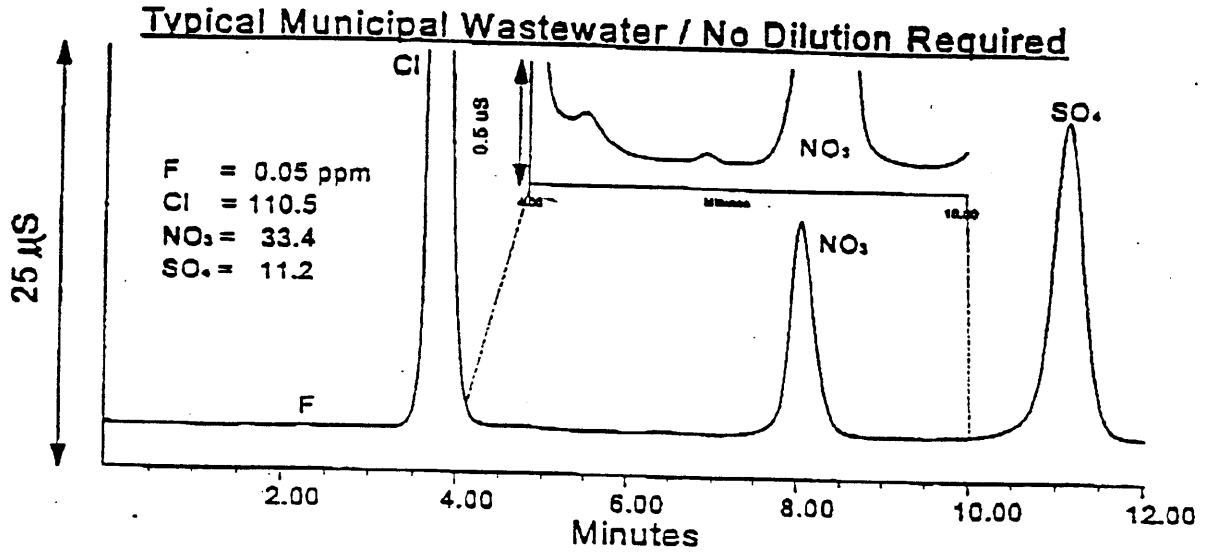
Fluoride = 5	Nitrite = 25	Nitrate = 25	Sulfate = 25
Chloride = 10	Bromide = 25	Phosphate = 75	

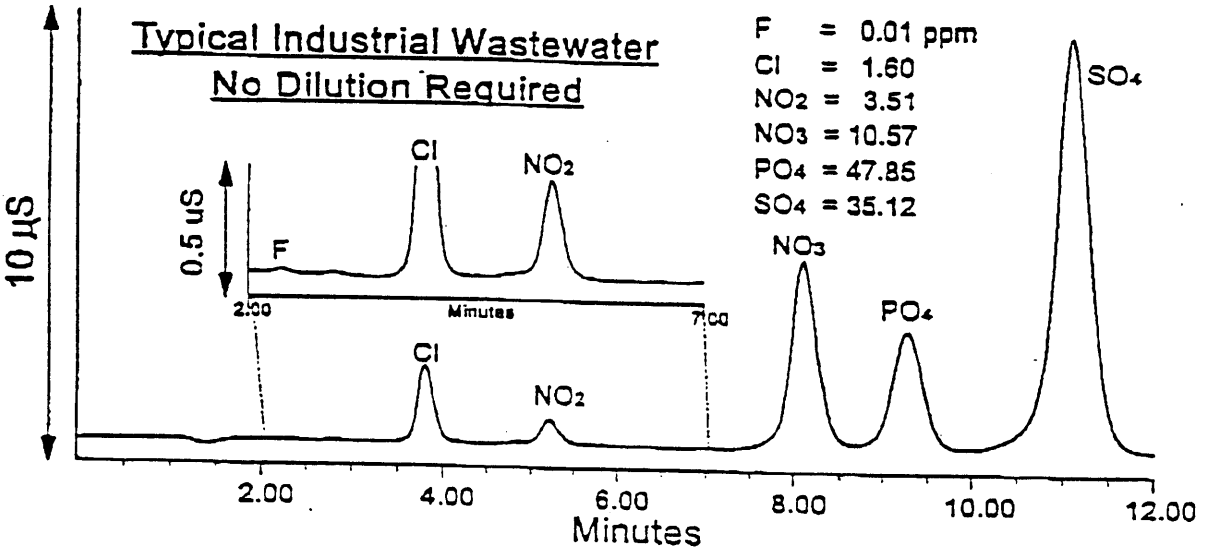
Lower detection limits can be obtained using a 250 µL injection. See Examples of Use for representative chromatogram.

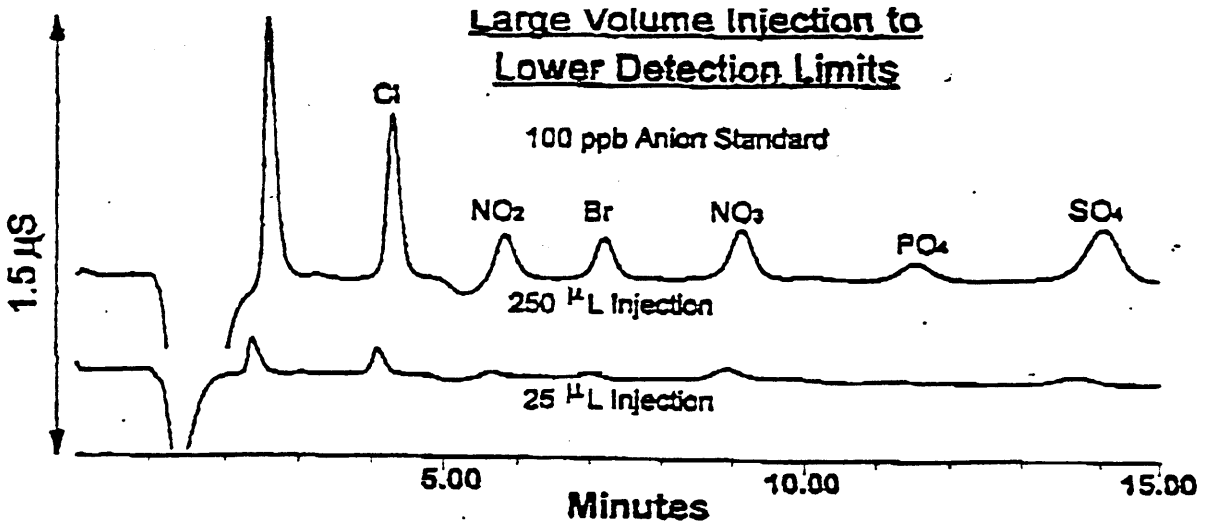
Use of Direct UV Detection:

Many anions are UV active in the range of 205 nm to 214 nm, such as NO_2, Br, and NO_3, and the use of direct UV detection provides a greater degree of detector selectivity. Refer to Waters Anion Analysis Method using Borate / Gluconate for details.


Typical Drinking Water
No Dilution Required
F = 0.39 ppm
Cl = 25.76
NO3 = 1.51
SO4 = 7.67
30 µS
0.13 uS
0.35 uS
F
Cl
NO3
SO4
5.00
10.00
14.00
Minutes


Typical Municipal Wastewater / No Dilution Required
F = 0.05 ppm
Cl = 110.5
NO3 = 33.4
SO4 = 11.2
25 µS
0.5 uS
F
Cl
NO3
SO4
2.00
4.00
6.00
8.00
10.00
12.00
Minutes


Typical Industrial Wastewater
No Dilution Required
F = 0.01 ppm
Cl = 1.60
NO2 = 3.51
NO3 = 10.57
PO4 = 47.85
SO4 = 35.12
10 µS
0.5 uS
F
Cl
NO2
NO3
PO4
SO4
2.00
4.00
6.00
8.00
10.00
12.00
Minutes

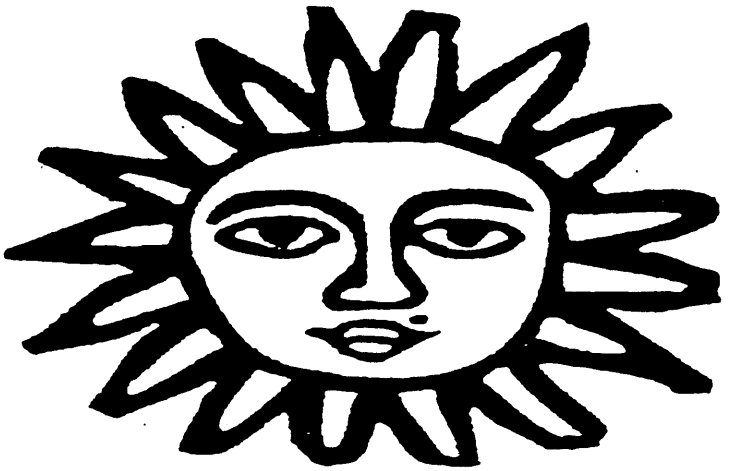


Stock Reagent Preparation:

100 mM Sodium Bicarbonate Solution: Dissolve 8.4 g of sodium bicarbonate ($NaHCO_3$) in a 1 liter volumetric flask with DI water, and fill to the mark with DI water. Store this solution in a capped plastic container at ambient temperature for up to 1 year.

100 mM Sodium Carbonate Solution: Dissolve 10.6 g of sodium carbonate (Na_2CO_3) in a 1 liter volumetric flask with DI water, and fill to the mark with DI water. Store this solution in a capped plastic container at ambient temperature for up to 1 year.

CHAMACOS
Home Inspection Form



CHAMACOS

Home Inspection Form

Date of Home Visit: __ __ / __ __ / __ __
MO DAY YR

Time Visit began: __ __ : __ __ AM/PM

Time Visit ended: __ __ : __ __ AM/PM

Collector who Completed Survey: _____ __ __
CODE

NOTE: There will be a separate form to document sample collection information (dust, molds, GPS and weather conditions).
The following equipment will be necessary to complete the home inspection: flashlight; compass; gloves; rolling tape measure; street map.

General suggestions:

A) Hiring a contractor to do some of the training – i.e. How do home inspection staff tell if something is vented outside, etc?

B) Hire a rep from Orkin or another extermination company to train staff to look for evidence of roaches, infestations, etc. Arthur Slater of UCB Pest Management Services has agreed to do the training (643-8079).

C) Kathleen will get a copy of the roach-extermination video used in the asthma study – how to look for stains, evidence of roaches, etc.

D.) Bring copy of study participant's signed consent form to home visit.

GENERAL INFORMATION

NOTE: All questions that are starred (*) should be asked to the respondent. Be sure to check the box provided if the question was answered by the respondent.

Check box if question is answered by respondent

1. Is this the house the mother lived in at the time of her baseline interview?
   No 0
   Yes 1

*2. Is this the house the mother lived in at the time she became pregnant? ☐
   No 0
   Yes 1

*3. Is this the house the mother lived in three months before becoming pregnant? ☐
   No 0
   Yes 1

HOME AND SURROUNDINGS

NOTE: All questions that are starred (*) should be asked to the respondent as well as noted by observation.

4. Which best describes the structure of the home?
   Detached home 1
   Duplex 2
   Multi-unit apt building 3
   Trailer/mobile home 4
   House in fields/camp 5
   Garage 6
   Hotel/motel 7
   Other, Specify _____ ... 8
   [CODE LATER]

5. Is there evidence of rubbish burning on the property?
   No 0
   Yes 1

6a. Are there farm animals living in the yard?
   No 0
   Yes 1

*6b Are there other animals living in the yard including pets? ☐
   No 0
   Yes 1
   Specify _____
   [CODE LATER]

7. Is there an outhouse/latrine?
   No 0
   Yes 1
   If yes, specify type _____
   [CODE LATER]

HOME AND SURROUNDINGS (CONT.)

Check box if answered by respondent

8. Choose one of the four photos that best describes the area near the front entrance:

All lawn or grass, well-maintained	1
All lawn or grass,overgrown/gone to seed	2
Mixed grass, dirt or sand	3
All dirt, sand or gravel	4
Hot top/ asphalt/ concrete	5

9. Choose one of the four photos that best describes the area near the back entrance:

No back entrance	0
All lawn or grass, well-maintained	1
All lawn or grass,overgrown/gone to seed	2
Mixed grass, dirt or sand	3
All dirt, sand or gravel	4
Hot top/ asphalt/ concrete	5

10. Choose one of the four photos that best describes the area near the side entrance:

No side entrance	0
All lawn or grass, well-maintained	1
All lawn or grass,overgrown/gone to seed	2
Mixed grass, dirt or sand	3
All dirt, sand or gravel	4
Hot top/ asphalt/ concrete	5

*11. Which door is most frequently used to enter the home? ☐

Front	1
Back	2
Side	3
DK	9

12. Is there a doormat next to the front door?

Yes-indoor	1
Yes-outdoor	2
Both indoor and outdoor	3
No mat	4

13. Is there a doormat next to the back door?

Yes-indoor	1
Yes-outdoor	2
Both indoor and outdoor	3
No mat	4
No back door	5

14. Is there a doormat next to the side door?

Yes-indoor	1
Yes-outdoor	2
Both indoor and outdoor	3
No mat	4
No side door	5

15. What is the condition of the mat(s) next to the door most frequently used to enter the home?

No doormat	0
Not worn	1
Worn	2
Extremely worn	3

ROOM BY ROOM ASSESSMENT

Instructions: Perform the following room-by-room assessment. Ask permission before entering each room. If you cannot access a given room, record 'not seen' in 16. Before leaving each room, make sure that you complete the "Room-Specific" questions that begin toward the end of the grid on page 6.

**** *NOTE: Establish guidelines for completing form if they only have one room that serves as kitchen and living room, etc.*

		Kitchen Area	Living Area	Mother's Bedroom	Bathroom number 1	Bathroom number 2
16.	Does this house have this type of room? [IF NO, SKIP TO NEXT COLUMN IN GRID]	No..... 0 Yes..... 1 Not seen... 2	No..... 0 Yes..... 1 Not seen... 2	No..... 0 Yes..... 1 Not seen... 2	No..... 0 Yes..... 1 Not seen... 2	No..... 0 Yes..... 1 Not seen... 2
17.	Is there a musty odor in this room?	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1
18.	Are there any windows that can be opened?	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1
19.	Is there any visible mold/mildew?	No...(21)... 0 Yes..... 1	No...(21)... 0 Yes..... 1	No...(21)... 0 Yes..... 1	No...(21)... 0 Yes..... 1	No...(21)... 0 Yes..... 1
20	Is there mold present on:					
	Walls	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2
	Ceiling	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2
	Window frame or coverings	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2
	Sink	None..... 0 Minimal..... 1 Extensive... 2	N/A	N/A	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2
	Tub/shower	N/A	N/A	N/A	None..... 0 Minimal..... 1 Extensive... 2	None..... 0 Minimal..... 1 Extensive... 2

ROOM BY ROOM ASSESSMENT (CONT.)

		Kitchen Area	Living Area	Mother's Bedroom	Bathroom number 1	Bathroom number 2	Check box if answered by respond.
21.	What types of window coverings are in this room? (drapes = heavier fabric or floor-length coverings) [CIRCLE ALL THAT APPLY]	No window 1 No coverings 1 Shades..... 1 Curtains.... 1 Drapes.... 1 Blinds..... 1	No window 1 No coverings 1 Shades..... 1 Curtains.... 1 Drapes.... 1 Blinds..... 1	No window 1 No coverings 1 Shades..... 1 Curtains.... 1 Drapes.... 1 Blinds..... 1	No window 1 No coverings 1 Shades..... 1 Curtains.... 1 Drapes.... 1 Blinds..... 1	No window 1 No coverings 1 Shades..... 1 Curtains.... 1 Drapes.... 1 Blinds..... 1	
22.	What type of floor covering is in the room? (hard = tile, linoleum, slate) [CIRCLE ALL THAT APPLY]	Wood..... 1 Hard Surface..... 1 Carpet..... 1 Other..... 1	Wood..... 1 Hard Surface..... 1 Carpet..... 1 Other..... 1	Wood..... 1 Hard Surface..... 1 Carpet..... 1 Other..... 1	Wood..... 1 Hard Surface..... 1 Carpet..... 1 Other..... 1	Wood..... 1 Hard Surface..... 1 Carpet..... 1 Other..... 1	
23a.	Carpet type? [CIRCLE ALL THAT APPLY] Thickness:	None..(24). 1 Area..... 1 Wall to wall 1 Level loop 1 Shag 2 In-between 3	None..(24). 1 Area..... 1 Wall to wall 1 Level loop 1 Shag 2 In-between 3	None..(24). 1 Area..... 1 Wall to wall 1 Level loop 1 Shag 2 In-between 3	None..(24). 1 Area..... 1 Wall to wall 1 Level loop 1 Shag 2 In-between 3	None..(24). 1 Area..... 1 Wall to wall 1 Level loop 1 Shag 2 In-between 3	
*23b	Does the carpet appear to be new (< 1 year)?	< 1 year..... 0 ≥ 1 year..... 1	< 1 year..... 0 ≥ 1 year..... 1	< 1 year..... 0 ≥ 1 year..... 1	< 1 year..... 0 ≥ 1 year..... 1	< 1 year..... 0 ≥ 1 year..... 1	☐
23c.	Are there stains/ discoloration or dirt, or evidence of wear?	Stains..... 0 1 Not worn... 0 Slightly worn... 1 Extremely worn... 2	Stains..... 0 1 Not worn... 0 Slightly worn... 1 Extremely worn... 2	Stains..... 0 1 Not worn... 0 Slightly worn... 1 Extremely worn... 2	Stains..... 0 1 Not worn... 0 Slightly worn... 1 Extremely worn... 2	Stains..... 0 1 Not worn... 0 Slightly worn... 1 Extremely worn... 2	
*24a	Is there a room air conditioner or fan?	None 0 Room AC 1 Fan.. 2 Used seasonally. 3	None 0 Room AC 1 Fan.. 2 Used seasonally. 3	None 0 Room AC 1 Fan.. 2 Used seasonally. 3	None 0 Room AC 1 Fan.. 2 Used seasonally. 3	None 0 Room AC 1 Fan.. 2 Used seasonally. 3	☐
*24b	[IF AC = YES], Is it a swamp cooler?	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	☐

ROOM BY ROOM ASSESSMENT (CONT.)

		Kitchen Area	Living Area	Mother's Bedroom	Bathroom number 1	Bathroom number 2
25.	Are there any potted house plants in this room? (DO NOT INCLUDE FRESH CUT FLOWERS)	No..... 0 Yes..... 1 Number:_	No..... 0 Yes..... 1 Number:__	No..... 0 Yes..... 1 Number:__	No..... 0 Yes..... 1 Number:__	No..... 0 Yes..... 1 Number:__
26.	Is there any evidence of mice, rats or other rodents (such as droppings, traps, poison) in room?	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1
27.	Is there any evidence of cockroaches (i.e. living or dead roaches, stains, roach motels) in this room?	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1
28a	Is there rotting wood around inside of window frames?	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2
28b	Is there peeling paint around inside of window frames?	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2	No..... 0 Yes..... 1 No window 2
29a	Is there rotting wood anywhere else in the room?	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1
29b	Is the peeling paint anywhere else in the room?	No..... 0 Yes..... 1	No..... 0 Yes..... 1	No..... 0 Yes..... 1 [Skip to 35]	No..... 0 Yes..... 1 [Skip to 39]	No..... 0 Yes..... 1

KITCHEN ONLY

30.	Are any of the following present in the kitchen?	
30a.	Plumbing leaks	No ..... 0 Yes 1
30b.	Overflowing trash	No ..... 0 Yes 1
30c.	Dirty dishes in sink/on stove	No ..... 0 Yes 1
30d.	Greasy stove	No ..... 0 Yes 1

KITCHEN ONLY (CONT.)

31.	Is there a refrigerator?	No ..... 0 Yes 1
32a.	Type of range top?	None ..... 0 Gas ..... 1 Electric ..... 2 Hot Plate 3
32b.	Is the range pilot burning? [IF GAS RANGE TOP]	No ..... 0 Yes 1
33a.	Type of oven?	None ..... 0 Gas ..... 1 Electric 2
33b.	Is the oven pilot burning? [IF GAS OVEN]	No ..... 0 Yes 1
34a.	Is there a vented fan over the stove?	No .....(35)..... 0 Yes 1
34b.	Is the fan in good working order?	No ..... 0 Yes 1

MOTHER'S ROOM ONLY

		Mother's Bedroom
35.	Is there a mite impermeable cover on the mattress?	No ..... 0 Yes ..... 1 No mattress..... 2
36.	Is there a mite impermeable cover on the pillow(s)?	No ..... 0 Yes ..... 1 No pillow..... 2
37.	Is the pillow filled with down?	No ..... 0 Yes ..... 1 No pillow..... 2
38.	Is there a down comforter on the bed?	No ..... 0 Yes 1

BATHROOM(S) ONLY

		Bathroom Number 1	Bathroom Number 2
39.	Is this a full or half-bath? (Full = toilet, sink and shower or tub Half = only toilet and sink)	Full ..... 1 Half .....(41)..... 2	Full ..... 1 Half .....(41)..... 2
40a	Is there an overhead fan?	No.....(41)..... 0 Yes..... 1	No.....(41)..... 0 Yes..... 1
40b	Is the fan in good working order?	No..... 0 Yes..... 1	No..... 0 Yes..... 1
40c	Is it vented to the outside?	No..... 0 Yes..... 1	No..... 0 Yes..... 1

OVERALL INTERIOR OF THE HOME

Check box if answered by respondent

41. How many rooms are in the home? [INCLUDE KITCHEN BUT NOT BATHROOM(S) OR HALLS]
1 1
2 2
3..... 3
4 4
5 or more 5

42. Is there electricity inside the home?
No 0
Yes..... 1

*43a Do they have/use any type of air cleaning devices? ☐
No .....(44)..... 0
Yes 1

*43b How often is the air cleaning device used? ☐
Daily 1
At least once per week 2
At least once per month 3
Less than once per month 4
DK 9

43c Who is the Manufacturer?
[Is this question necessary?]
_____

43d What is the Model Number?
[Is this question necessary?]
_____

43e Is it a HEPA cleaner?
No 0
Yes 1

44. Are there any signs of smoking in the home? (cigarette or cigar butts, pipes, ash-trays)
No 0
Yes 1

TRACKING- IN OF DUST AND DIRT

Check box if answered by respondent

*45a Is there a vacuum in the home?
No .....(46)..... 0
Yes ..... 1 ☐

*45b What is the Brand and Model of the vacuum?
Brand : _____ __ __
[CODE LATER]
Model: _____ __ __
[CODE LATER] ☐

*45c Does the vacuum have a powered brush?
No 0
Yes ..... 1 ☐

45d Does the vacuum have a HEPA filter?
No .....(46)..... 0
Yes 1

45e [IF WRITTEN ON THE FILTER]
What is the HEPA filter rating?
_____ __ __
[CODE LATER]

FURNITURE

46. How many pieces of stuffed or cloth-covered chairs, couches, or love seats are in the home?
0 0
1-2 1
3-5 2
more than 5 3

BASEMENT

47. Is there a crawl space under the home?
No 0
Yes 1

48. Is there a basement in the home?
No .....(51)..... 0
Yes 1

49. Are there signs of dampness in the basement (musty odor; visible water, mold, or mildew; discoloration on walls, damp carpets or furniture)?
No 0
Yes 1
No access to basement..... 2

Specify type _____

*50. At other times of the year, does water get into this basement?
No 0
Yes ..... 1 ☐

INDOOR AIR

Check box i answered b respondent

*51. What is the principal heat source during heating season?

Do not use heat	00
Gas	01
Electric	02
Oil	03
Kerosene	04
Coal	05
Wood	06
Solar	07
Propane	08
Steam radiators	09
DK	99

52a. Is there a wood stove or fireplace?

No (53)	0
Yes	1

*52b How often is it used?

Never (53)	0
Every day or night	1
A few times a week	2
A few times a month	3
DK	9

52c. What do you usually burn in the wood stove or fireplace?
[CIRCLE ALL THAT APPLY]

Wood	1
Artificial logs	1
Paper	1
It's a gas fireplace	1
Other	1
Specify _____	
DK	1

*53. Are there any unvented kerosene or gas heaters? (include portable or wall units, under floor)

No	0
Yes	1

*54a Is there a water heater?

No (55)	0
Yes	1

*54b Is it a gas water heater?

No (55)	0
Yes	1

*54c Where is the gas water heater located?

Kitchen	1
Bathroom	2
Other main part of home	3
Garage	4
Basement	5
Separate room for laundry	6
Outside (enclosed)	7
Other _____	8
[CODE LATER]	

INDOOR AIR (CONT.)

				Check box if answered by respondent
55a.	Is there central air conditioning in the home?	No ....(56)....	0	
		Yes	1	
55b.	Is the AC a swamp cooler?	No	0	
		Yes	1	
*56a	Is there a vaporizer in the home?	No ....(57)....	0	☐
		Yes	1	
*56b	How often is the vaporizer used?	Daily	1	☐
		Weekly	2	
		At least once per month	3	
		Less than once per month	4	
		DK	9	
*57a	Is there a humidifier in the home?	No ....(58)....	0	☐
		Yes	1	
*57b	How often is the humidifier used?	Daily	1	☐
		Weekly	2	
		At least once per month	3	
		Less than once per month	4	
		DK	9	
58a.	Is there a clothes dryer?	No ....(59)....	0	
		Yes	1	
58b.	Is it vented to the outside?	No	0	
		Yes	1	
58c.	Is it a gas dryer?	No	0	
		Yes	1	

GARAGE

Check box if answered by respondent

59a.	Is there a garage?	No ....(62)....	0
		Yes	1
59b.	Is the garage attached?	No	0
		Yes	1
*59c	Is the garage used to park a car?	No	0 ☐
		Yes	1
60.	Is there a doormat between the garage and house?	No	0
		Yes	1
61.	Does the garage have strong chemical or fuel odors?	No	0
		Yes	1

TRAFFIC DENSITY

*62.	On average, how often do trucks or buses pass through on the road adjacent to the home?	Never	0 ☐
		≤1 per day	1
		2-10 per day	3
		>10 per day	4
		N/A	8
*63.	Is daytime traffic noise so loud that you often close the windows?	No	0 ☐
		Yes	1

HOME PESTICIDES

Instructions: Perform this section last and be sure to use gloves when handling pesticide containers. DO NOT handle any open or leaking containers.

*64a. List the brand name of all pesticides found in the home or yard.	*64b. Type of chemical/ used for	64c. CAS #	64d. EPA Registration #	64e. Active ingredient	64f. Weight/ volume of container	*64g. Storage location (CODE)	*64h. Most recent pesticide application (CODE)	*64i. How often is pesticide applied? (CODE)	64j. Was question answered by respondent NO=0 YES=1	64k. Comments

64g. Storage location (CODE)
1) Garage
2) Shed (detached from house)
3) Bathroom
4) Kitchen
5) Utility room/closet
6) Other _____

64h. Most recent pesticide application (CODE)
1) Within last week
2) One to 4 weeks ago
3) One to 3 months ago
4) Three to 6 months ago
5) Six to 12 months ago

64i. How often is pesticide applied? (CODE)
1) Daily
2) Weekly
3) At least once per month
4) Less than once per month

LOCATION AND SURROUNDING OF HOME

Instructions: Perform the following assessment based on visual inspection.

	How far is the home from the nearest:			Indicate direction relative to home (N=1, S=2, E=3, W=4, SW=5, NW=6, NE=7, SE=8, DK=9)	Check box if answered by respondent
*65.	Major road or highway with >= 4 lanes of traffic	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(66).....	4		
*66.	Busy road or highway with 2-3 lanes of traffic	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(67).....	4		
*67.	Unpaved road	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(68).....	4		
*68.	Manufacturing / food processing facility	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(69).....	4		
*69.	Garbage dump	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(70).....	4		
*70.	Golf course	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(71).....	4		
*71.	Recreational park/ school grounds	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile.....(72).....	4		
*72.	Gas station or autobody / repair shop	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(73).....	4		

LOCATION AND SURROUNDING OF HOME (CONT.)

	How far is the home from the nearest:			Indicate direction relative to home (N=1, S=2, E=3, W=4, SW=5, NW=6, NE=7, SE=8, DK=9)	Check box if answered by respondent
*73.	Large parking lot for stores	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile.....(74).....	4		
*74.	Warehouse w/ diesel trucks	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(75).....	4		
*75.	Agricultural field	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(76).....	4		
*76.	Fields that are <u>currently</u> tarped	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile .....(77).....	4		
*77.	Stream from agricultural run-off	< 50 ft, 15 meters	1	____	☐
		50 – 200 ft, 15-60 meters.....	2		
		200 ft- ¼ mile.....	3		
		> ¼ mile.....(78).....	4		

78. Is there any discharge into the yard (i.e., discolored water/mud etc. from sewage, household waste, etc.)?

No 0
Yes 1

Specify type _____
[CODE LATER]

79. Are there any bad or unpleasant odors outside?

No .....(81)..... 0
Yes 1

80. Describe the intensity of each odor:

Garbage

None 0
Light 1
Moderate 2
Strong 3
Very strong..... 4
Overpowering 5

Sewage

None 0
Light 1
Moderate 2
Strong 3
Very strong..... 4
Overpowering 5

SURROUNDING OF HOME (CONT.)

80. Describe the intensity of each odor (cont.):

	Chemical	None ..... 0 Light ..... 1 Moderate ..... 2 Strong ..... 3 Very strong..... 4 Overpowering 5
What does it smell like? _____		
	Diesel	None ..... 0 Light ..... 1 Moderate ..... 2 Strong ..... 3 Very strong..... 4 Overpowering 5
	Smoke	None ..... 0 Light ..... 1 Moderate ..... 2 Strong ..... 3 Very strong..... 4 Overpowering 5
	Pesticides	None ..... 0 Light ..... 1 Moderate ..... 2 Strong ..... 3 Very strong..... 4 Overpowering 5
	Other _____	None ..... 0 Light ..... 1 Moderate ..... 2 Strong ..... 3 Very strong..... 4 Overpowering 5

81. Are any of the following trees and plants present in the yard? [SHOW PHOTOS]

Coastal live oak	No	1
	Yes	2
	DK	9
Acacia	No	1
	Yes	2
	DK	9
Pine	No	1
	Yes	2
	DK	9
California black oak (all trees)	No	1
	Yes	2
	DK	9
Sagebrush (weed)	No	1
	Yes	2
	DK	9
Bermuda	No	1
	Yes	2
	DK	9
Rye grasses	No	1
	Yes	2
	DK	9

OVERALL CONDITION OF THE HOME (HOUSEKEEPING)

NOTE: This question is to be completed after leaving the home, at the end of the survey.

82. Describe the quality of the housekeeping.

Extremely poor housekeeping, no recent cleaning, lack of organization, greasy cooking area, clutter throughout	1
Not as bad as 1, but unless some attention is given to housekeeping, could become a "1"	2
The "average" level of housekeeping. Periodic cleaning occurs	3
Above average, clean without much clutter	4
"Good housekeeping award". Organized, nothing out of place	5

QUALITY OF THE HOME VISIT

83. Who was the main respondent during the walkthrough?

- Mother of baby 1
- Father of baby 2
- Other household member 3
- Other non-household member 4
  - Specify _____
- N/A 8

84. Who was the secondary respondent during the walkthrough?

- Mother of baby 1
- Father of baby 2
- Other household member 3
- Other non-household member 4
  - Specify _____
- N/A 8

85. How responsive was the participant to the home visit?

- Not responsive at all 1
- Generally responsive 2
- Extremely responsive 3

NOTE: Environmental collector, please respond to the following question or write "N/A".

86. Are there any concerns about the study participant's health or exposures that you would like to report?

_____

_____

_____

_____

_____

_____

87. List below any questions asked by respondent that you were not able to answer: